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Contents

A Specific Inhibitor for Human Desoxyribonuclease and an Inhibitor of the Lupus Erythematosus Cell Phenomenon from Leucocytes. NATHANIEL B. KURNICK, LAWRENCE I. SCHWARTZ, SANFORD PARISER, AND STANLEY L. LEE 193

Cerebral Blood Flow and Oxygen Consumption in Hyperthyroidism Before and After Treatment. LOUIS SOKOLOFF, RICHARD L. WECHSLER, RENWARD MANGOLD, KENT BALLS, AND SEYMOUR S. KETY 202

Observations on the Volume of Distribution of Inulin in Anuric Subjects. JOHN T. FINKENSTAEDT, MAURICE P. O'MEARA, AND JOHN P. MERRILL 209

The Value of Needle Biopsy in the Chemical Estimation of Liver Lipids in Man. BARBARA H. BILLING, HAROLD J. CONLON, DAVID E. HEIN, AND LEON SCHIFF 214

A Comparison of the Effects of *l*-nor-Epinephrine, Synthetic *l*-Epinephrine and U.S.P. Epinephrine upon Cerebral Blood Flow and Metabolism in Man. WILLIS SENSENBACH, LEONARD MADISON, AND LAMAR OCHS 226

The Effect of an Arteriovenous Fistula on Renal Hemodynamics and Electrolyte Excretion. FRANKLIN H. EPSTEIN, ROBERT S. POST, AND MARION MCDOWELL 233

The Effects of Quiet Standing on Solute Diuresis. ALLAN V. N. GOODYER AND DONALD W. SELDIN 242

The Glycogen Content of Human Leukocytes in Health and in Various Disease States. WILLIAM N. VALENTINE, JAMES H. FOLLETTE, AND JOHN S. LAWRENCE 251

Metabolic and Renal Studies in Chronic Potassium Depletion Resulting from Overuse of Laxatives. WILLIAM B. SCHWARTZ AND ARNOLD S. RELMAN 258

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The prophylactic administration of BICILLIN to these patients did not cause a noticeable change in pharyngeal flora. There was no increase in monilia in the throat or stools of 77 patients. Seventy-seven dental extractions were performed on 38 patients during the period of this study and no case of subacute bacterial endocarditis developed.

Penicillin hypersensitivity was encountered in four patients. In each case the symptoms were transitory and did not require interruption of prophylaxis. No alteration in the peripheral blood cell count, results of urinalysis, blood urea nitrogen or blood sugar levels was noted.

There was no instance of a recurrence of rheumatic fever in any patient receiving regular doses of BICILLIN.

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*Abstracted from Stollerman, G.H., and Rusoff, J.H.: Prophylaxis against group A streptococcal infections in rheumatic fever patients, J.A.M.A. *150*:1571 (Dec. 20) 1952.

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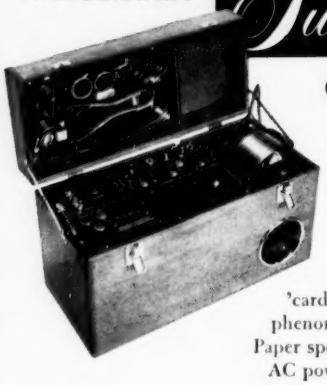

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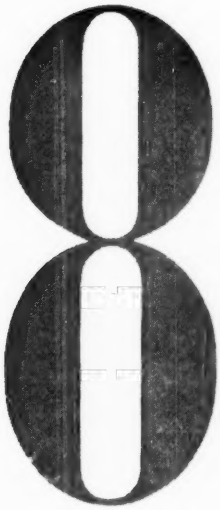

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Vol. 11, No. 4

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CONTENTS

		Page
1.	**The fibrous structure of normal and abnormal skin.** By R. E. Tunbridge, R. N. Tattersall, D. A. Hall, W. T. Astbury and R. Reed	315
2.	**The basis of the erythrocyte sedimentation rate.** By J. Hardwicke and J. R. Squire	333
3.	**The renal response to respiratory alkalosis.** By S. W. Stanbury and A. E. Thomson	357
4.	**Some evidence in favour of the secretion of water and solutes by the kidney.** By Margaret H. Roscoe	375
5.	**Effects of methyl testosterone and deoxycortone on electrolytes.** By Paul Fourman	387
6.	**Experimental potassium depletion in man.** By D. A. K. Black and M. D. Milne	397
7.	**Hypoferraemia in the human subject: the importance of diurnal hypoferraemia.** By J. C. S. Paterson, D. Marrack and H. S. Wiggins	417
8.	**The utilization of folic acid from natural sources.** By G. H. Spray	425
9.	**Correlation of skin temperature and circulatory changes in muscle and subcutaneous tissue of the hand during trunk heating.** By R. Harris, A. J. Martin and H. S. Williams	429
10.	**The iodine uptake of the human thyroid throughout the menstrual cycle and in pregnancy.** By E. E. Pochin	441
11.	**Secretion of radioiodine in digestive juices and milk in man.** By A. J. Honour, N. B. Myant and E. N. Rowlands	447

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A SPECIFIC INHIBITOR FOR HUMAN DESOXYRIBONUCLEASE AND AN INHIBITOR OF THE LUPUS ERYTHEMATOSUS CELL PHENOMENON FROM LEUCOCYTES [1]

By NATHANIEL B. KURNICK, LAWRENCE I. SCHWARTZ, SANFORD PARISER,[2] AND STANLEY L. LEE

(From the Department of Medicine, Tulane University School of Medicine, New Orleans, Louisiana; and the Hematology and Pathology Laboratories of the Mount Sinai Hospital, New York, N. Y.)

(Submitted for publication September 29, 1952; accepted December 3, 1952)

In speculating upon the probable role of desoxyribonuclease (DNase) in the depolymerization of desoxyribonucleic acid (DNA), which characterizes (1) the "lupus erythematosus (L.E.) cell" (2), we have suggested (3), as have Klemperer and his associates (4), that an increased serum enzyme activity, potentiation of an intracellular DNase, or destruction of an intracellular inhibitor of DNase by a serum factor, might be responsible. In a previous communication (5) we reported evidence which excludes the serum DNase from responsibility for the phenomenon. Studies on the interaction of leucocytes and serum, in an approach to the alternative possibilities, disclosed the presence of a specific intracellular inhibitor of human serum DNase and an inhibitor of the "L.E. cell" phenomenon (6, 7). In the present paper, we shall report upon some of the characteristics of these factors.

I. SERUM DNASE INHIBITION

A. Preparation of leucocyte extracts

Leucocytes (WBC) were prepared with the aid of Bovine fraction I from heparinized blood as previously described (5) or by centrifugation at 0 to 2°C of fresh "ACD" blood [3] (120 ml. of 1.47 per cent dextrose, 1.32 per cent sodium citrate, 0.48 per cent citric acid plus 480 ml. whole blood) followed by collection of the WBC layer and repeated washing at 0 to 2°C with 0.14 M NaCl until a good separation of WBC from erythrocytes was obtained. The latter technique was used with large volumes of human blood (2500-3000 ml.).

Extracts of WBC were prepared by either of two methods:

(a) Suspensions of 24×10^6 or more WBC per ml. in 0.14 M NaCl or heparinized plasma were lysed by repeated freezing in acetone-solid CO_2 mixtures followed by thawing by immersion in a 37°C water bath. Complete disintegration of cells was accomplished by freezing and thawing four times. An aliquot was clarified by centrifugation at $2700 \times G$ (measured at center of tube) for 30 minutes in a Sorvall angle centrifuge. The material was stored at −20°C.

(b) Approximately 12×10^9 fresh WBC were suspended in 30 ml. 0.14 M NaCl at 0 to 2°C and blended in a micro Waring Blendor equipped with an ice jacket (8), slightly modified by the addition of a rubber diaphragm seal for the ice jacket, to permit pouring from the Blendor without contamination of the material by the ice-saline mixture in the jacket, for 10 minutes (sufficient for complete fragmentation of the cells, as demonstrated by microscopic examination). The homogenate was centrifuged for 15 minutes at $430 \times G$. The supernatant, representing the bulk of the cytoplasmic material and some soluble nuclear components, was labelled "cytoplasmic fraction." This was separated by centrifugation for 40 minutes at $2700 \times G$ into a "cytoplasmic supernatant" and "cytoplasmic sediment." The sediment obtained from the whole homogenate at $430 \times G$ was washed repeatedly with saline in order to obtain a clean "chromosomal fraction" and fractionated into nucleohistone (DNH) and "residual chromosomes" after Mirsky and Ris (9, 10). From DNH, purified desoxyribonucleic acid (DNA) was prepared according to Mirsky and Pollister (11). All fractions were resuspended in 0.14 M NaCl to reconstitute, approximately, the original concentration (12×10^9 WBC in 30 ml.). All procedures were carried out at 0 to 2°C. Upon completion of the fractionation, sodium ethyl mercuri-thiosalicylate was added to a final concentration of 1:100,000, and the material was stored at −20°C. A pale pink color was imparted to the cytoplasmic fraction and the supernatant obtained therefrom

[1] This work was aided by grants to Tulane University School of Medicine (N. B. K.) from the National Heart Institute, U.S.P.H.S. (No. H-714), the American Heart Association, American Cancer Society (recommended by the Committee on Growth, National Research Council) and the Life Insurance Medical Research Fund, and to the Mount Sinai Hospital from the Life Insurance Medical Research Fund and A. A. List.

[2] Anna Ruth Lowenberg Fellow in Hematology, Mount Sinai Hospital.

[3] We are indebted to Dr. J. W. Davenport and Mr. John Gavey of the Medical Department of the New Orleans Regional Blood Center, American Red Cross, for generous supplies of human blood.

by the hemoglobin of the RBC which remained entrapped in the WBC layers.

B. Inhibition of serum DNase

Each of the WBC extracts prepared by the freezing-thawing method was tested for DNase activity by the methyl green method (3) as modified for serum (12). In no case was any DNase activity detected under these conditions of ionic strength and pH. Tests for DNase activities under other conditions (13–17) were not made, since these would not interfere with our experiments. Tests for DNase inhibition against human serum were performed by substituting the fraction to be tested for part or all of the 3 ml. saline used in diluting the substrate (12). Simultaneous observations on the inhibition of the "L.E. cell" phenomenon by each of the fractions are reported below. The observations on the two types of inhibition parallel each other closely. Rarely, preparations which had previously been found to contain inhibitor, failed to inhibit the "L.E. cell" phenomenon and only weakly inhibited human serum DNase. As will be seen, these failures were probably due to proteolysis (autolysis), whereas the discrepancies result from the greater sensitivity of the DNase system.

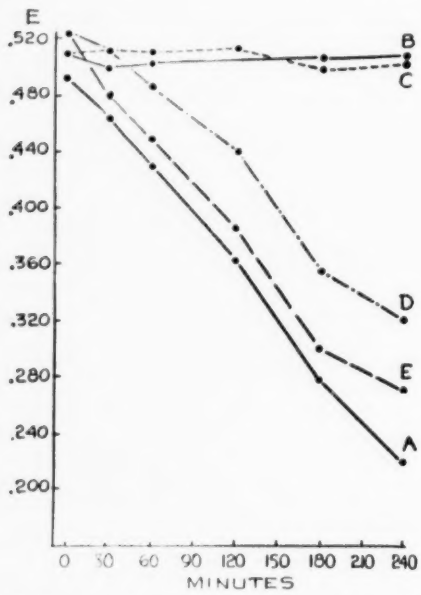

FIG. 1. HUMAN SERUM DNASE INHIBITION BY HUMAN LEUCOCYTE (WBC) EXTRACTS

Each point represents optical density of 3 ml. substrate-inhibitor-serum mixture added to 0.5 ml. 0.33 M sodium citrate after incubation for intervals indicated at 37°C. Each 19 ml. of the incubated mixture contains 1 ml. serum and 1 ml. of the following saline WBC extracts: A. None (1 ml. 0.14 M saline), B. Supernatant from 6×10^8 frozen and thawed cells, C. "Cytoplasmic supernatant," D. Unwashed "cytoplasmic sediment," E. Chromosome fraction.

Inhibition of human serum DNase was observed with the frozen and thawed extracts, both before and after centrifugation, with the total "cytoplasmic fraction," and the "cytoplasmic supernatant" prepared from blended WBC. Neither the "cytoplasmic sediment" nor the "chromosomal fraction" (nor fractions prepared therefrom) demonstrated inhibition (Fig. 1).

The relationship between the amount of inhibitor present and the degree of inhibition is apparently not linear (Fig. 2), and resembles the curve reported by Zamenhof and Chargaff (13) for their yeast DNase inhibitor.

C. Physico-chemical properties of inhibitor

Prolonged dialysis against frequent changes of 0.14 M saline at 0°C did not alter the inhibitory activity of extract fractions. No loss of inhibition resulted from heating at 56°C for 2 hours (both unbuffered and buffered at pH 7.5 with trimethylol-amino-methane buffer [18]). Heating to 100°C in a boiling water bath for 30 minutes completely destroyed all inhibitory effect. The addition of citric acid to bring the final concentration to 1 per cent, followed by dialysis against saline at 0°C (to remove citrate), resulted in loss of inhibitory activity by "cytoplasmic supernatant." This was probably a pH effect, since treatment of inhibitors prepared by freezing and thawing cells into saline with 0.1 N NaOH or 0.06 N HCl also destroyed their inhibitory effect. However, 0.00025 N HCl (as used in the pepsin digestion described below) did not affect inhibition.

Since the citrate ion is a potent inhibitor of DNase, it seemed possible that traces of this ion from the ACD solution might have been responsible for the inhibition of serum DNase by the "cytoplasmic supernatant." The following observations, previously noted, militate against this possibility:

(a) Potent inhibitors were prepared from heparinized blood, (b) 100°C destroyed the in-

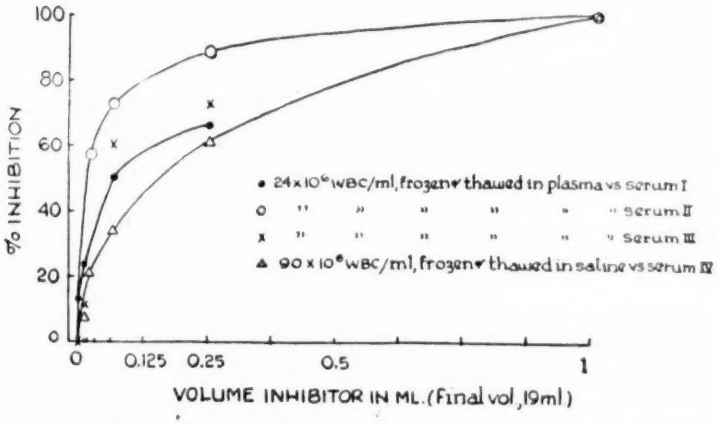

FIG. 2. DEGREE OF INHIBITION OF SERUM DNASE VS. VOLUME (CONCENTRATION) OF INHIBITOR (19 ML. FINAL VOLUME OF SUBSTRATE-INHIBITOR-SERUM MIXTURE CONTAINING 1 ML. SERUM)

hibition, (c) 1 per cent citric acid, NaOH, and HCl destroyed inhibition, and (d) dialysis did not reduce inhibition. This possibility was further excluded by a direct test:

To 20 ml. of a "whole chromosome" suspension, and of a "cytoplasmic sediment" suspension, 5 ml. of "ACD" solution were added. After an hour at room temperature, the material was dialyzed against 0.14 M NaCl at 0–2°C for 24 hours, with frequent changes of the bath. No inhibition of human serum DNase was detected with either material before or after the treatment with "ACD."

D. Effects of enzymes

"Cytoplasmic supernatant" inhibitors were incubated for 2 hours at 37°C with an equal volume of solutions containing 3 mg. per ml. of the following enzymes [4] in 0.01 M pH 7.5 trimethylol-aminomethane buffer (18): ribonuclease, trypsin, chymotrypsin, papain, and yeast protease. Ribonuclease was also used at 56°C for 2 hours. Pepsin was similarly used, but dissolved in water (final pH 4.7) or in 0.0005 N HCl. Two ml. of each mixture was tested for inhibitory effect against human serum DNase (final volume 19 ml. as in [12]). Controls consisted of the inhibitor incubated with the solvent (i.e., without enzyme) and of the enzyme incubated with saline (i.e., without inhibitor). The inhibitor controls showed unimpaired inhibitory action and the enzyme controls had no effect on serum DNase activity except in the case of yeast protease, which, alone, revealed DNase activity, and produced an additive DNase activity with sera. All of the proteolytic enzymes completely destroyed the inhibition of serum DNase, while ribonuclease was without effect upon the inhibitor.

E. Permeability of cell membrane to inhibitor

Since WBC were found to contain an inhibitor for human serum DNase, it appeared possible that the very low serum DNase values commonly encountered in normal sera (12) might result from exposure of the serum to the WBC of the blood before separation, particularly if separation was not carried out promptly. Freshly clotted blood was allowed to stand at room temperature and at 37°C. Samples of sera were removed at intervals from 15 minutes to 6 hours after collection. Similar experiments were performed with heparinized blood. No change in DNase activity was observed over this period. After 24 hours at 37°C, there was moderate reduction of DNase activity, but this was no greater than was noted when separated serum was allowed to stand for the same interval. The fact that no significant amount of inhibitor "leaked" into autologous serum or plasma was also supported by the observation that in no case was inactive serum found to inhibit the DNase of an active serum. However, 0.14 M saline, allowed to stand in contact with isolated WBC at 0 to 2°C for 24 to 48 hours, ex-

[4] Crystalline enzymes obtained from General Biochemicals, Inc., Chagrin Falls, Ohio.

TABLE I

Inhibition of serum desoxyribonuclease by various cell types

Diagnosis	No. of cells per ml. of saline	Approx. No. cells × 10^4 per ml.				Inhibition, per cent
		Lympho-cytes	Granulo-cytes	Mono-cytes	Blast cells	
1) Lymph. Leukemia	20 × 10^6	14	6	0	0	32
2) Normal	35 × 10^6	15	18	2	0	39
3) Monocytosis	170 × 10^6	17	68	85	0	41
4) Normal	174 × 10^6	75	84	15	0	66
5) Ac. Myel. Leukemia	240 × 10^6	5	35	0	200	56
6) Chronic Myel. Leukemia	300 × 10^6	2	290	0	8	36

tracted a significant amount of inhibitor from them, as demonstrated by inhibition of serum DNase by the supernatant. Furthermore, 60 × 10^6 WBC washed four times with 0.14 M NaCl, then frozen and thawed into 1 ml. of the test serum, produced only slight inhibition of DNase, while unwashed WBC, similarly treated, produced complete inhibition.

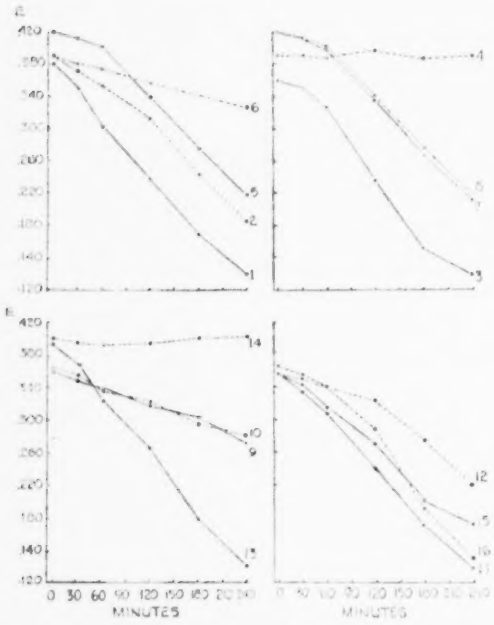

FIG. 3. INHIBITION OF VARIOUS DESOXYRIBONUCLEASES BY HUMAN WBC "CYTOPLASMIC SUPERNATANT"

Solid line represents activity of substrate-serum alone; dotted line represents activity of substrate-serum mixture plus 1 ml. "cytoplasmic supernatant" (final volume 19 ml.). *1, 2.* Dog serum, *3, 4.* Rabbit serum, *5, 6.* Guinea pig serum, *7, 8.* Cat serum, *9, 10.* Rat serum, *11, 12.* Hamster serum, *13, 14.* Human serum, *15, 16.* Bovine pancreatic DNase.

F. Inhibitor preparations from different cell types

The presence of inhibitor in different types of cells was studied by freezing and thawing in 0.14 M saline, WBC isolated from a normal person, a patient with post-splenectomy monocytosis (40,000 WBC per cmm. capillary blood, 50% monocytes), a patient with chronic myelogenous leukemia, one with lymphosarcoma and lymphatic leukemia (70% lymphocytes), and one with acute myelogenous leukemia. The effect of 0.5 ml. of the supernatant from each was tested against 1 ml. of a single serum sample (Table I).

The data suggest that lymphocytes may be richer in inhibitor than granulocytes (cf. 1 and 6) and that monocytes may be poor in inhibitor (cf. 3 and 4). The relationship found between the inhibition by 35 × 10^6 and by 174 × 10^6 normal WBC appears consistent with the data in Fig. 2. Immature leucocytes appear to be richer in inhibitor than mature WBC (cf. 5 and 6). Caution is necessary in interpreting these observations, however, since completeness of inhibitor extraction may vary among the cell types.

G. Species specificity of inhibitor

Nine different inhibitors prepared from human WBC were tested against the sera of dog (diluted 3:4 with saline), cat (diluted 3:100), rabbit (diluted 1:2), hamster (undiluted), guinea pig (diluted 3:4), rat (undiluted), and mouse (undiluted). Dilutions were selected so that 1 ml. of the diluted serum approximated the DNase activity of active human serum (about 140 × 10^{-4} units per ml. serum [see (3) for the definition of DNase unit]). Bovine pancreatic DNase (19)[5] was similarly tested in a final concentration of 0.005 μg. per ml. The results of a typical experi-

[5] Worthington Laboratories, Freehold, N. J.

TABLE II

Enhanced susceptibility of washed leucocytes to "L.E. cell" transformation

L.E. serum dilution (initial)	Unwashed WBC				Washed (4×) WBC			
	"Globs"	"L.E. cells"	Rosettes	Total	"Globs"	"L.E. cells"	Rosettes	Total
Undil.	2	2	1	5	2	3	3	8
1:2	0	1	1	2	2	2	3	7
1:4	0	0	0	0	2	2	2	6
1:8	0	0	0	0	1	0	1	2
1:16	0	0	0	0	0	0	0	0

ment are shown in Fig. 3. As indicated by the curves, only the human and rabbit DNase were completely inhibited. There was moderate inhibition of guinea pig and hamster serum DNase, questionable inhibition of canine DNase, and no inhibition of rat, cat, and mouse serum and bovine pancreatic DNase.

II. "L.E. CELL" PHENOMENON INHIBITOR

A. Quantitation of "L.E. cell" phenomenon

Sera with "L.E. phenomenon activity" (L.E. serum) were the same as those reported previously (5). Leucocyte (WBC) "buttons" of 10–15 × 10^6 WBC were prepared as in that paper (5). These WBC "buttons" were used as substrate for the L.E. phenomenon without further treatment or after washing four times by resuspension in about 7 ml. isotonic saline and re-centrifugation. The "buttons" were suspended in 0.5 ml. saline (or inhibitor to be tested), 0.5 ml. of L.E. serum (diluted with normal serum) was added, and the mixture incubated at 37°C for 1 hour. The WBC were then sedimented by centrifugation for 5 minutes at low speed, smeared upon glass slides, fixed in absolute methyl alcohol for 5 minutes, and stained in dilute Giemsa solution for 30 minutes. The stained smears of a given experiment (with appropriate controls) were examined by a single observer who (to insure objectivity) did not know the labelling code. Each of the three morphological manifestations of the L.E. phenomenon (a. classical "L.E. cell" with single large inclusion, or "droplet cell" with several small inclusions, b. rosettes, c. "globs" [5]) was graded from 0 to 4 on the basis of frequency of occurrence.

B. Increased susceptibility of washed WBC to "L.E. cell" transformation

It was regularly found that washed WBC were more susceptible to "L.E. cell" transformation than were unwashed WBC, as measured both by the intensity of the "L.E. cell" phenomenon at any given dilution of L.E. serum and by the titer to which the L.E. serum would tolerate dilution before loss of demonstrable L.E. activity (Table II). To exclude the possibilities (*a*) that the removal of the native plasma or the heparin and fibrinogen included therein and (*b*) that the traumatization of the WBC by repeated centrifugation had caused their greater susceptibility to the "L.E. cell" transformation, two controls were added. In one, the washed WBC were resuspended in their original plasma, re-centrifuged, and tested. No diminution in the enhancement of the "L.E. cell" phenomenon produced by washing was observed. In the second control, unwashed WBC were resuspended in the original plasma and re-centrifuged

TABLE III

*Inhibition of "L.E. cell" phenomenon by leucocyte homogenate**

L.E. serum dilution (initial)	Control (normal plasma)				Inhibited (WBC homogenate)**			
	"Globs"	"L.E. cells"	Rosettes	Total	"Globs"	"L.E. cells"	Rosettes	Total
Undil.	2	3	3	8	2	1	1	4
1:2	1	2	3	6	1	1	1	3
1:4	1	2	1	4	1	1	1	3
1:8	1	1	1	3	0	0	0	0
1:16	1	1	0	2	0	0	0	0
1:32	1	0	0	1	0	0	0	0
1:64	0	0	0	0	0	0	0	0

* Washed WBC (15 × 10^6) plus 0.5 ml. normal plasma or WBC homogenate plus 0.5 ml. L.E. serum (diluted with normal serum).

** WBC homogenate consists of 40 × 10^6 WBC per ml. frozen and thawed in normal plasma.

and resuspended four times. Such unwashed, but repeatedly resuspended and re-centrifuged WBC revealed no increased susceptibility to "L.E. cell" transformation. Controls in which normal sera, with and without DNase activity, were substituted for active L.E. sera revealed no "L.E. cell" induction in either washed or unwashed WBC.

C. "L.E. cell" phenomenon inhibition

The probability that the increased susceptibility of washed WBC to "L.E. cell" transformation was due to the partial removal from the WBC of inhibitor was confirmed by replacing the inhibitor.

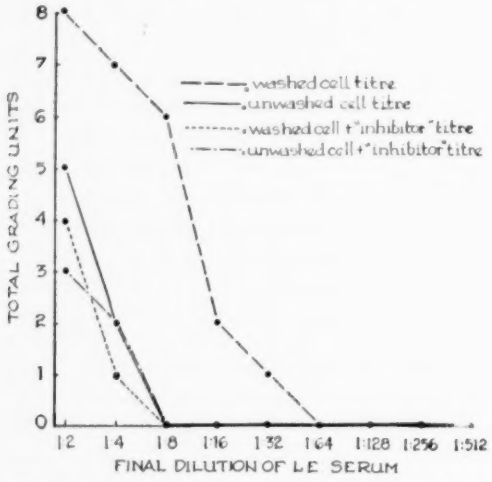

FIG. 4. EFFECT OF SUPERNATANT FROM 24×10^6 WBC PER ML. FROZEN AND THAWED IN NORMAL SERUM ON INTENSITY OF "L.E. CELL" PHENOMENON AT SEVERAL DILUTIONS OF L.E. SERUM (IN NORMAL SERUM)

The washed WBC were resuspended in a WBC homogenate or a WBC fraction (see Part I, A for description of fractions). The whole homogenates, and each of the fractions which contained DNase inhibitor ("cytoplasmic fraction," "cytoplasmic supernatant"), but not those without DNase inhibitor activity (chromosomal fraction, cytoplasmic sediment, DNH, DNA, histone, "residual chromosomes"), reduced the intensity of the "L.E. cell" phenomenon in washed WBC (Table III). Slight inhibition of "L.E. cell" transformation of unwashed WBC was also demonstrated (Fig. 4).

In order to test the relationship between inhibition of the "L.E. cell" phenomenon and the concentration of the inhibitor, an experiment was performed in which the inhibitor, a homogenate of 24×10^6 WBC per ml. plasma (frozen and thawed), was serially diluted with normal plasma. The control contained normal plasma in place of the inhibitor. The data are presented in Fig. 5, and the percentage inhibition in Fig. 6. The similarity between Fig. 6 and Fig. 2 is apparent. It should be borne in mind that the percentage inhibitions shown in Fig. 6 represent the ratios of the total grading units of the inhibited and uninhibited experiments. This is only a very rough estimate of the degree of inhibition, therefore. Since the

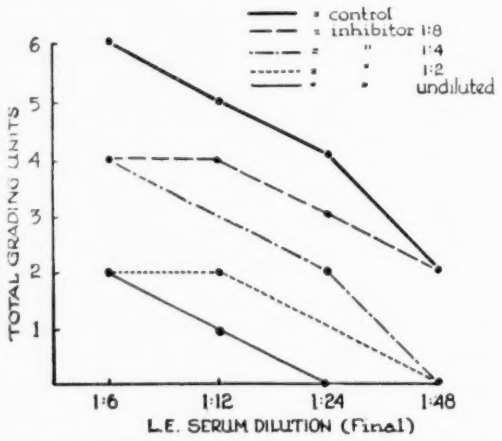

FIG. 5. EFFECT OF CONCENTRATION OF INHIBITOR ON THE INTENSITY OF THE "L.E. CELL" PHENOMENON PRODUCED IN WASHED WBC BY SEVERAL DILUTIONS OF L.E. SERUM

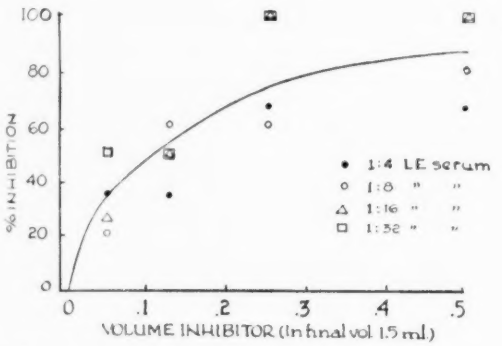

FIG. 6. DEGREE OF INHIBITION OF "L.E. CELL" PHENOMENON VS. VOLUME (CONCENTRATION) OF INHIBITOR (FROM DATA IN FIG. 5)

final volume is approximately 1.5 ml. in the "L.E. cell" experiments, whereas it is 19 ml. in the DNase experiments, the greater sensitivity of the DNase system to the inhibitor is obvious.

D. Physico-chemical properties of "L.E. cell" phenomenon inhibitor

Like the DNase inhibitor, the "L.E. cell" phenomenon inhibitor was found to be unaffected by a temperature of 56°C for 30 minutes, but was destroyed at 100°C for 15 minutes. Similarly, dialysis did not impair the inhibition of the "L.E. cell" phenomenon. After repeated use over a period of weeks, which required bringing the material to room temperature for short intervals and refreezing, inhibition of both the serum DNase and of the "L.E. cell" phenomenon became progressively less effective. This was presumed to be due to proteolysis, since, under the conditions described in Part I, D, the "L.E. cell" inhibitor was also destroyed by proteolytic enzymes.

TABLE IV

Effect of anions on "L.E. cell" phenomenon

Salt	Concentration	Result*			
		Undil.**	1:4	1:16	1:64
Sodium Chloride	0.14 M	10	6	3	1
Sodium Arsenate	0.2 M	10	4	0	0
Potassium Oxalate	0.2 M	6	1	0	0
Sodium Citrate	0.2 M	5	2	1	0

* Total grading score. Maximum is 12 (4 each for "L.E. cells," rosettes, "globs")

** Dilutions indicated are those of the L.E. serum (diluted with normal serum)

E. Effect of magnesium ion binding anions on "L.E. cell" phenomenon

Since intracellular DNase is thought to play a part in the production of the "L.E. cell" phenomenon, anionic DNase inhibitors were tested. These ions are thought to act by binding Mg^{++}, which is required for optimal DNase activity. The WBC buttons were suspended in 0.5 ml. of the electrolyte solution, followed by 0.5 ml. L.E. serum. Thus, the final concentration of the salt was approximately one-third that indicated in Table IV, which presents the observed results. Definite inhibition by citrate, oxalate, and arsenate, in order of decreasing effect was apparent.

DISCUSSION

The experimental data demonstrate the presence in human leucocytes of an inhibitor for human serum DNase, and an inhibitor of the "L.E. cell" phenomenon.

These inhibitors do not permeate a cellophane membrane, are destroyed by heating to 100°C but are stable at 56°C, are stable at − 20°C for several months and at 0–2°C for at least several days. They are destroyed by a number of proteolytic enzymes, but not nucleolytic enzymes. Extremes of pH also destroy the DNase inhibitor. Efforts at purification and further physico-chemical characterization of the factor or factors are in progress.

The DNase inhibitor reveals species specificity to some extent (only in the rabbit is the same degree of inhibition obtained as in the human). It may be suggested that the specificity is due to the requirement for a hypothetical serum co-factor. However, inhibitors prepared by freezing and thawing WBC in their own plasma, which would contain the hypothetical co-factor, reveal the same specificity as do saline extracts. Thus, the specificity is probably a function of differences in DNase from various species. Immunologic differences in DNases have already been demonstrated by McCarty (20).

That the inhibitors are present in the leucocytes is apparent from the method of preparation. The only other elements regularly present are thrombocytes. However, potent inhibitors were obtained from the WBC of a patient with severe thrombocytopenia. The inhibitors are found in the supernatant upon rupturing the cells in saline. Although this fraction is ordinarily regarded as cytoplasmic, it undoubtedly includes some proteins of nuclear origin. The cytological localization of the inhibitors is, therefore, unsettled.

Desoxyribonuclease inhibition by cell extracts has also been reported by others. The inhibitor of yeast DNase which Zamenhof and Chargaff (13), obtained from yeast, resembles the inhibitor in WBC described in this paper in its species specificity (for yeast), in its instantaneous effect upon the enzyme activity, and in its protein nature, but is less stable (destroyed at 56°C in 5 minutes). Dabrowska, Cooper and Laskowski (21) and Cooper, Trautmann and Laskowski (22) prepared an inhibitor of bovine DNase from several

avian and mammalian organs. These inhibitors were also proteins and were very unstable. This instability interferes with the separation of excess DNase activity from the inhibitor content of cell extracts since both DNase activity and inhibitor are destroyed at 56°C. Thus, these inhibitors differ from ours both in lack of species specificity and in stability.

Henstell and Freedman (*23, 24*) have recently reported on the preparation of an inhibitor of bovine DNase from leucocytes by the dilution of whole blood 1 : 100 or 1 : 1000 with water. Their inhibitor appears to be very similar to that of Laskowski's group in its non-specificity and instability. It differs from that reported by us, because it is potent in very great dilution and is less, rather than more, abundant in immature cells. It is apparent that we failed to observe the inhibitor reported by these authors because of its instability, since several hours were required for the preparation of the inhibitor in leucocytes, and it was not tested on the day of preparation.

Bernheimer and Ruffier (*25*) isolated a specific DNase inhibitor from streptococci. This inhibitor, however, differs from that described here in being destroyed by ribonuclease but not by proteolytic enzymes, thus indicating its non-protein nature. It appears, then, that we have observed a new inhibitor from mammalian tissues, most closely resembling that described by Zamenhof and Chargaff (*13*) in yeast.

Close parallelism between the inhibitor of serum DNase described by us and the inhibitor of the "L.E. cell" phenomenon suggest their identity. The two activities are located in the same WBC fraction. They show the same heat stability and are non-dialyzable. Complete correspondence is not observed in the intensity of the effect, in that more nearly complete inhibition of serum DNase than of the "L.E. cell" phenomenon is usually obtained. This, however, may not indicate lack of identity of the inhibitors, since the intracellular DNase is probably less accessible to inhibitor added *in vitro*, and the intra- and extracellular DNases may not be identical in susceptibility to the inhibitor. Proof of the identity of the inhibitors must await their further purification.

Since the evidence suggests the identity of the intracellular DNase inhibitor and the inhibitor of the "L.E. cell" phenomenon, it is probable that the depolymerization of DNA which characterizes the "L.E. cell" is secondary to a derangement of the intracellular DNase-DNase inhibitor system. This abnormality is not primary in the cell, since it can be induced in leucocytes from a normal individual (*26*) by serum from a patient with systemic lupus erythematosus. We suggest that the L.E. plasma factor (*27*), which itself has no DNase activity (*5*), causes the release of the intracellular DNase from inhibition. The inhibition of the "L.E. cell" phenomenon by anionic DNase inhibitors, as well as by an extract which contains a naturally occurring DNase inhibitor, supports this hypothesis.

This hypothesis led to the development of a "sensitized" "L.E. cell" test, using WBC, which had been washed to remove inhibitor. False positive tests are not produced, perhaps because unbroken WBC contain some inhibitor even after repeated washing. This was demonstrated for the DNase inhibitor. Thus, the L.E. serum factor is still required to elicit the phenomenon.

The fact that mature granulocytes show greater susceptibility to the "L.E. cell" transformation than lymphocytes (*28*) may be due to the apparent greater inhibitor content of the latter noted in our studies (Table I). Likewise, the lesser susceptibility of immature granulocytes may be attributed to their greater DNase inhibitor content as compared with mature leucocytes.

Observations on the DNase inhibitor content of various tissues, normal and pathological, and at various stages of development are in progress. Some observations on lymphocytes, blasts, and granulocytes have already been referred to above. The possible role of a specific inhibitor of DNase in cellular division, growth, senescence, and the development of tumors, which suggests itself, is under further investigation.

SUMMARY

1. An inhibitor for human serum DNase has been observed in human leucocytes. It is a protein, soluble in saline, stable at 56°C. Tested against several species, it is equally effective only against rabbit serum DNase and has little or no influence on some others. In its stability and specificity it differs from other DNase inhibitors previously reported.

2. An inhibitor for the "L.E. cell" phenomenon has been demonstrated in leucocytes. Evidence which suggests its identity with the DNase inhibitor is presented.

3. A sensitive "L.E. cell" test using washed WBC is described.

4. A mechanism for the "L.E. cell" phenomenon is discussed.

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CEREBRAL BLOOD FLOW AND OXYGEN CONSUMPTION IN HYPERTHYROIDISM BEFORE AND AFTER TREATMENT[1]

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It is generally believed that the alterations in total body oxygen consumption which accompany functional disturbances of the thyroid gland reflect widespread metabolic changes in all cells and tissues (1, 2). Numerous studies have identified some of the tissues that participate in the over-all disturbance of metabolic rate. For example, *in vitro*, kidney, liver, heart, and skeletal muscle tissues of animals previously treated with thyroid substance or thyroxine display increased oxygen consumption (3–7), whereas kidney, liver and muscle of thyroidectomized animals show lower than normal oxygen consumptions (5, 8). In man, Myers, using the bromsulphalein method, found the splanchnic oxygen consumption in hyperthyroidism to be elevated out of proportion to the total body metabolic rate, although the splanchnic blood flow remained essentially unchanged (9).

In the case of the metabolic rate of the brain, *in vitro* studies have yielded controversial results. Cohen and Gerard (10) have found considerably higher than normal rates of oxygen consumption in minced brain tissue of rats made hyperthyroid by the administration of thyroid substance. Similar results have been obtained by MacLeod and Reiss (11) who administered thyrotropic hormone following hypophysectomy. On the other hand, no such increases were found by Spirtes (12) or by Gordon and Heming (6), and Fazekas and associates (13) could find no difference from normal in the cortical oxygen consumption of both hyperthyroid and hypothyroid rats.

Studies of the effect of desiccated thyroid substance on the cerebral metabolic rate of human subjects suffering from cretinism were made by Himwich and co-workers (14). Using the thermoelectric flow recorder and cerebral arterio-venous oxygen differences, they found marked increases in cerebral blood flow and cerebral oxygen consumption following thyroid administration. Only relative changes could be measured by this method, however. By means of the nitrous oxide method, quantitative determinations of cerebral blood flow and oxygen consumption in patients with hyperthyroidism have failed to demonstrate any significant differences from normal young men (15–17). On the other hand, in myxedema, a markedly reduced cerebral blood flow and metabolism and an elevated cerebral vascular resistance have been reported by Scheinberg and associates (18). Recently, Madison, Sensenbach and Ochs (19) have reported the results of a similar study on four hyperthyroid and four myxedematous subjects, the latter studied both before and after therapy. Using as normal controls the values obtained when the myxedematous subjects were made euthyroid by treatment, they failed to find in untreated myxedema the decrease in cerebral metabolic rate described by Scheinberg, although the circulatory findings were essentially the same. In their hyperthyroid group, however, in contrast to the previous studies of Sokoloff and his coworkers (15, 16) and Scheinberg (17), Madison and coworkers (19) found the cerebral blood flow to be significantly elevated compared to that in treated myxedema whereas cerebral oxygen consumption was normal.

In view of the discrepancies in the previous studies of cerebral blood flow and metabolism in hyperthyroidism, it was felt that further investigation was indicated. This paper presents the results obtained in a larger group of hyperthyroid patients than previously reported. Repeated studies in the same patients when they were in the euthyroid state following therapy served as controls. This provides not only a more valid basis

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for comparison than that hitherto employed but also provides information on the effect of therapy on the blood flow and metabolism of the brain in hyperthyroidism. Moreover, data on blood pH, carbon dioxide content, carbon dioxide tension, and hemoglobin concentration are presented because of the known effect of these blood constituents on cerebral blood flow (*20*).

METHOD

The cerebral blood flow (CBF) of eleven untreated patients with hyperthyroidism, whose ages varied from 21 to 58, was determined by means of the method of Kety and Schmidt (*21*). The same studies were repeated on seven patients when they were considered to be euthyroid following medical or surgical treatment. Diagnosis was based on clinical history, physical examination, basal metabolic rate, the level of protein-bound iodine in the serum, and in some cases, the rate of I^{131} uptake by the thyroid gland. Mean arterial blood pressure (MABP) was measured with a damped mercury manometer connected to the femoral arterial needle. Blood oxygen and carbon dioxide contents were determined by the manometric method of Van Slyke and Neill (*22*). Arterial hemoglobin concentration was measured in the Evelyn photometer according to the modification of the method of Evelyn and Malloy (*23*). Blood pH was measured anaerobically at room temperature by means of the glass electrode and potentiometer and was corrected to 37° C., according to the factors of Rosenthal (*24*). Calculations of the cerebral oxygen consumption (CMR_{O_2}), cerebral vascular resistance (CVR), and cerebral respiratory quotient (RQ) were made as described previously (*21*). Blood carbon dioxide tension (pCO_2) was computed by means of the nomograms of Peters and Van Slyke (*25*).

Each patient was in the basal state at the time of the study. Immediately following the procedure for cerebral blood flow determination, the total body metabolic rate was measured with a clinical Benedict-Roth metabolism recording apparatus. The basal metabolic rate determination was performed after the blood flow procedure so as to take into account at least some of the effects of excitement associated with the latter measurement, thus permitting a more valid comparison between cerebral and total body metabolic rates.

RESULTS

The data are presented in Tables I and II. Included for comparison are the results obtained in 11 normal young men, similarly studied by the same group of investigators over approximately the same period of time. No significant difference from the normal values previously reported by Kety and Schmidt (*21*) were found in the normal group. Values obtained by Fazekas and his associates (*26*) in a normal group whose age more closely approximated that of our patients, are also presented in Table I.

In the seven patients studied before and after treatment, the marked fall in basal metabolic rate from + 57 per cent to + 7 per cent ($p < 0.01$) following therapy was not accompanied by any significant change in CMR_{O_2}. Also, the mean value for CMR_{O_2} of 3.5 cc. O_2 per 100 g. per minute in the untreated group did not differ significantly from that of the normal young men and was identical to the value obtained by Fazekas and his coworkers, in a normal group of comparable age (*26*). There was no significant correlation between basal metabolic rate and cerebral oxygen consumption in both the treated and untreated groups ($r = -0.06$, $p > 0.1$; $r = -0.18$, $p > 0.1$ respectively). The mean cerebral blood flow of 69.0 cc. of blood per 100 g. per minute in untreated hyperthyroidism significantly exceeded the value of 54.2 in Fazekas' group (*26*) and the value of 54.8 obtained in the normal young men ($p < 0.05$). Following treatment, cerebral blood flow decreased to 60 cc. per 100 g. per minute. This value did not differ significantly from the cerebral blood flow values obtained before therapy, in the normal young men, or in the comparable age group described by Fazekas, Alman, and Bessman (*26*). The high mean values for mean arterial blood pressure, 104 mm. Hg, agreed closely with the value of 112 mm. Hg obtained by Fazekas in his group of normal subjects of comparable age (*26*). On the other hand, the cerebral vascular resistance before treatment was 1.63 mm. Hg per cc. per 100 g. per minute, a normal value for normal young men but significantly decreased for this age group ($p < 0.05$) (*26*). This can be attributed to the anemia indicated by the low hemoglobin concentration of 11.35 g. per cent. That cerebral vascular resistance and hemoglobin concentration are interrelated in hyperthyroidism is suggested by the significant correlation between these 2 factors ($r = + 0.68$; $p < 0.02$). Treatment was not accompanied by any significant change in mean arterial blood pressure, cerebral vascular resistance or hemoglobin concentration. Arterial and cerebral venous oxygen contents were significantly lower than normal both before and after treatment ($p < 0.001$) as a result of the low hemoglobin concentrations. Arterial and cerebral venous pH, CO_2

TABLE I

CEREBRAL CIRCULATION AND METABOLISM IN HYPERTHYROIDISM BEFORE AND AFTER TREATMENT

Subj.	Age	Sex	B. M. R.		MABP mm. Hg		CBF cc/100g/min.		CVR mm. Hg/cc/100g/min.		$(A-V)_{O_2}$ Vol. %		CMR_{O_2} $cc_{O_2}/100g/min.$		Cerebral RQ	
			I	II	I	II	I	II	I	II	I	II	I	II	I	II
Hyperthyroidism - 11 cases before treatment and 7 cases after treatment.																
M.L.	44	F	+57%	---	108	-----	62	---	1.7	---	4.51	----	2.8	---	0.80	---
A.H.	23	F	+51%	---	102	-----	80	---	1.4	---	4.55	----	3.8	---	0.81	---
E.T.	45	F	+57%	---	135	-----	82	---	1.6	---	5.90	----	4.9	---	0.97	---
E.D.	21	F	+12%	---	101	-----	79	---	1.3	---	4.86	----	3.8	---	0.87	---
E.M.	49	F	+30%	+28%	92	134	50	55	1.8	2.4	5.54	5.67	2.8	3.1	0.83	0.93
E.W.	58	F	+65%	+15%	97	122	66	82	1.5	1.5	4.24	4.95	2.8	4.1	0.86	0.94
E.H.	42	F	+69%	+28%	77	99	98	81	0.8	1.2	4.14	4.13	4.0	3.3	1.00	1.04
W.E.	52	M	+49%	+25%	102	120	38	34	2.7	3.5	7.97	6.05	3.0	2.1	0.91	0.94
D.W.	49	F	+88%	- 6%	108	120	63	64	1.7	1.9	4.87	3.68	3.1	2.4	0.82	1.01
H.J.	36	F	+46%	-35%	142	122	59	60	2.4	2.0	6.18	6.13	3.6	3.7	1.05	1.04
E.P.	24	F	+51%	- 6%	79	93	79	44	1.0	2.1	5.10	6.37	4.0	2.8	0.86	0.93
MEAN	40.3	-	+52.3%	+ 7%	103.9	115.7	69.0	60.0	1.63	2.09	5.26	5.28	3.51	3.07	0.89	0.98
S.E.	± 3.6	-	± 6.0	±8.95	±6.0	±5.95	±5.2	±6.7	±0.17	±0.28	±0.34	±0.40	±0.20	±0.27	±0.02	±0.02
p*	----	-	<0.01		>0.05		>0.5		>0.05		>0.7		>0.4		<0.05	
Normal Young Males - 11 Cases																
MEAN	21.8	-	----	----	86.5	-----	54.8	---	1.68	---	6.25	----	3.34	---	0.92	---
S.E.	±0.6	-	----	----	±1.7	-----	±4.3	---	±0.13	---	±0.40	----	±0.23	---	±0.03	---
p†	<0.001	-	----	----	<0.02	-----	<0.05	---	>0.8	---	>0.05	----	>0.5	---	>0.7	---
Normals of Comparable Age - 9 Cases (Fazekas, et al [26])																
MEAN	35	-	----	----	111.6	-----	54.2	---	2.08	---	----	----	3.49	---	----	---
S.E.	± 2.2	-	----	----	±5.8	-----	±2.8	---	± 0.11	---	----	----	±0.14	---	----	---
p‡	>0.2	-	----	----	>0.3	-----	<0.05	---	< 0.05	---	----	----	>0.9	---	----	---

I = Untreated Hyperthyroidism.
II = Treated Hyperthyroidism.

*Determined from the t value obtained by dividing the mean of the individual changes following therapy by its standard error.
†Determined from the t value obtained by dividing the difference between the means in untreated hyperthyroidism and in normal young men by the standard error of the difference between these means.
‡Determined from the t value obtained by dividing the difference between the means in untreated hyperthyroidism and in normal subjects of comparable age by the standard error of the difference between these means.

TABLE II

BLOOD CONSTITUENTS IN HYPERTHYROIDISM BEFORE AND AFTER TREATMENT

Subj.	Hemoglobin Concentration Grams %		Blood O_2 Content vol. % Arterial		Blood O_2 Content vol. % Int. Jugular		Blood CO_2 Content vol. % Arterial		Blood CO_2 Content vol. % Int. Jugular		Blood pH Arterial		Blood pH Int. Jugular		Blood CO_2 Tension mm. Hg Arterial		Blood CO_2 Tension mm. Hg Int. Jugular	
	I	II	I	II	I	II	I	II	I	II	I	II	I	II	I	II	I	II
Hyperthyroidism - 11 cases before treatment and 7 cases after treatment.																		
M.L.	11.31	-----	11.64	-----	10.13	-----	48.54	-----	52.13	-----	7.37	----	7.32	----	42	--	50	--
A.P.	9.60	-----	11.89	-----	7.34	-----	51.55	-----	55.24	-----	7.41	----	7.39	----	40	--	45	--
P.S.	13.27	-----	17.59	-----	11.66	-----	52.49	-----	58.25	-----	7.44	----	7.29	----	40	--	56	--
R.D.	12.00	-----	15.97	-----	11.11	-----	50.16	-----	54.36	-----	7.43	----	7.41	----	40	--	43	--
K.M.	11.51	14.34	15.34	17.50	9.80	11.83	48.37	50.63	52.89	55.27	7.35	7.40	7.29	7.33	42	41	56	58
E.R.	10.23	14.25	13.65	17.49	9.42	12.54	52.03	50.38	55.66	55.01	7.41	7.36	7.33	7.28	40	49	48	60
E.H.	10.07	12.50	13.88	15.53	9.74	11.20	51.30	47.94	55.46	52.26	7.39	7.35	7.34	7.30	42	44	50	52
M.R.	14.24	13.65	19.46	18.15	11.59	12.10	40.79	46.26	48.07	51.73	7.43	7.43	7.36	7.37	33	37	46	46
D.M.	10.60	10.80	12.60	14.02	7.73	10.34	57.11	58.52	61.12	62.22	7.38	7.32	7.34	7.28	42	56	50	64
R.J.	12.72	10.80	16.41	14.67	10.23	8.64	44.31	48.36	50.78	54.74	7.40	7.39	7.32	7.34	38	41	44	50
R.S.	10.64	11.22	13.88	16.10	8.78	9.73	44.64	36.71	49.00	42.55	7.40	7.38	7.32	7.34	36	32	47	39
MEAN	11.35	12.51	15.03	16.21	9.77	10.90	49.21	48.31	53.91	53.42	7.40	7.37	7.35	7.32	39.5	43.1	49.1	51.9
S.E.	±0.48	±0.60	±0.67	±0.59	±0.42	±0.61	±1.38	±2.44	±1.17	±2.21	±0.01	±0.01	±0.01	±0.01	±0.9	±3.0	±1.1	±3.2
P*	>0.1		> 0.1		> 0.05		> 0.9		> 0.4		> 0.1		> 0.3		> 0.1		> 0.3	
Normal Young Males - 11 Cases																		
MEAN	14.67	-----	19.44	-----	13.20	-----	47.45	-----	53.11	-----	7.39	----	7.34	----	41.3	---	51.6	---
S.E.	±0.60	-----	±0.49	-----	±0.53	-----	±0.73	-----	±0.80	-----	±0.01	----	±0.01	----	±0.9	---	±1.3	---
P†	<0.001	-----	<0.001	-----	<0.001	-----	>0.2	-----	>0.5	-----	>0.4	----	>0.4	----	>0.1	---	>0.05	---

I = Untreated Hyperthyroidism.
II = Treated Hyperthyroidism.

*Determined from the t value obtained by dividing the mean of the individual changes following therapy by its standard error.

†Determined from the t value obtained by dividing the difference between the means in untreated hyperthyroidism and in normal young men by the standard error of the difference between these means.

contents, and CO_2 tensions were all within normal limits both before and after treatment. Although the values for cerebral respiratory quotient both before and after treatment did not differ significantly from normal, therapy resulted in a significant increase from 0.89 to 0.98 ($p < 0.05$). The reasons for this change are obscure.

DISCUSSION

The finding of the same cerebral oxygen consumption in this hyperthyroid group both before and after effective therapy, as well as the lack of a significant difference between this cerebral oxygen consumption and that obtained from a normal group of comparable age and a normal group of young men, certainly suggests that cerebral metabolism is unaltered in hyperthyroidism. This is in agreement with previous observations in this disease (15–17, 19). However, cerebral blood flow was found to be significantly elevated in our hyperthyroid group whereas a normal cerebral blood flow was reported by Scheinberg (17). We are unable to explain this discrepancy. In our series, blood CO_2 tensions, oxygen saturations and pH, known regulators of cerebral circulation, were found to be normal whereas hemoglobin concentration was significantly reduced. In view of the significant correlation between hemoglobin concentration and cerebral vascular resistance in untreated hyperthyroidism, it appears that the anemia in our patients offers the best explanation for the low cerebral vascular resistance and, consequently, for the elevated cerebral blood flow. In the group studied by Scheinberg, anemia may not have been present, but absence of data on blood constituents precludes further speculation.

The normal cerebral blood flow and CMR_{O_2} and the greater than normal incidence of a low arterial-cerebral venous oxygen difference found by Scheinberg led him to postulate an increased extra-cerebral contamination of cerebral venous blood in hyperthyroidism (17). If, however, the original nitrous oxide method is employed, independent criteria for the recognition of extra-cerebral contamination are made available (21). Instead of drawing integrated arterial and internal jugular venous blood samples over the full ten minutes, as in Scheinberg's modification, nitrous oxide concentration-time curves for arterial and internal jugular venous blood are constructed on the basis of a number of blood samples taken during the ten minute period. From these curves, the value of $\frac{V_t \times 100}{\int_0^t (A - V)\, dt}$ from zero time to the end of each minute is calculated. When contamination is absent or insignificant, this function falls rapidly over the first seven minutes and then levels off, and its value over the full ten minutes is taken as the cerebral blood flow. If, on the other hand, the function continues to fall even at ten minutes, then contamination is present, and the rate of fall is an indication of its degree. These criteria apply to contamination with extra-cerebral venous blood, the type most commonly encountered. The remote possibility exists, however, that the contaminating blood may be "arterialized" as, for example, in case of arteriovenous shunts or unusually rapid blood flow through the extra-cerebral tissues. Any contamination of this type sufficient to affect significantly the calculated value obtained for cerebral blood flow would also noticeably alter the internal jugular nitrous oxide concentration-time curve. This curve would then rise more abruptly from zero, and the abrupt rise would be manifest in a disproportionately small integrated arterial-internal jugular nitrous oxide difference over the first minute. On the basis of these criteria, no greater evidence of either type of contamination was found among the hyperthyroid patients than in the treated patients, or normal subjects. It appears, therefore, at least in our group, that the low arterial-cerebral venous oxygen difference in hyperthyroidism is simply the result of an elevated cerebral blood flow in the presence of a normal oxygen consumption.

An explanation of the failure of the brain to participate in the increased total body metabolism in hyperthyroidism can at the present time be only speculative. Cerebral metabolism may be unaffected by the thyroid hormone because of an inability of the active principle to penetrate the blood-brain barrier. We are unaware, however, of any information concerning this problem.

Scheinberg (17), supported by Fazekas (13), has postulated that the "cerebral metabolism normally functions at close to its maximum rate and that a large part of the energy used by the brain goes to maintaining the structural and biochemical

organization of the brain." Supposedly, therefore, hyperthyroidism could not be expected to cause a measurable increase in cerebral oxygen consumption above the normal level. This hypothesis is questionable, in view of the recent observations of significantly elevated cerebral metabolic rates in response to infusions of synthetic l-epinephrine (*27*) and in moderate muscular exercise (*28*).

A more likely explanation for the normal oxygen consumption of the brain in hyperthyroidism is simply that the energy metabolism of the fully developed brain is of such a nature as to render it independent of the action of the thyroid hormone. The uniqueness of the cerebral metabolism is already indicated in the fact that the normal brain derives its energy almost exclusively from the utilization of carbohydrate (*29*, 30). Similarly, the testis, which is also believed to utilize only carbohydrate (31), has a normal oxygen consumption in hyperthyroidism (*6*). It appears then that the thyroid hormone, whose exact function in intermediary metabolism is still unknown, does not measurably alter the metabolic processes of those tissues which consume carbohydrate for energy almost exclusively. On the other hand, Fazekas (13) has observed that in brain tissue in which protein metabolism must be significant (e.g., the developing rat cortex), the oxygen consumption rises more rapidly to the normal adult level in induced hyperthyroidism than in the normal or hypothyroid states. Once fully developed to the adult level, however, when growth and protein metabolism become less significant, the oxygen consumption was found to be the same in the normal, hypothyroid and hyperthyroid groups (13). Since the thyroid hormone does influence the development of the brain (13), i.e., its structural and enzymatic proteins, it is likely that it is also involved in the maintenance of these systems in the mature brain. This effect in the mature brain, however, may be normally not detectable because of the low turn-over rate of these protein systems compared to that of the carbohydrates. When the thyroid hormone is insufficient adequately to maintain these systems, as, for example, in myxedema, then secondarily, the energy producing carbohydrate metabolism may be impaired and this reflected in a lowered cerebral oxygen consumption (14, 18).

SUMMARY

1. Studies of cerebral blood flow, cerebral vascular resistance, and cerebral oxygen consumption, as well as mean arterial blood pressure, hemoglobin concentration, blood gases, and blood pH, were made on eleven untreated hyperthyroid patients and then repeated on seven following treatment.

2. Cerebral oxygen consumption was entirely normal and was not significantly correlated with basal metabolic rate.

3. Comparison with the results of similar studies in normal individuals indicated a moderate elevation of cerebral blood flow in untreated hyperthyroidism probably because of an associated anemia. Mean arterial blood pressure was normal, but cerebral vascular resistance was low for this age group. Except for the low oxygen contents associated with a moderate anemia, blood gases were found to be within normal limits.

4. Treatment did not result in any significant changes except for the decrease of the basal metabolic rate to the normal level and an increase in the cerebral respiratory quotient.

5. Possible explanations for the failure of the brain to participate in the elevated total body metabolic rate are discussed. It is suggested that the gross energy metabolism of the brain, as reflected by the oxygen utilization, may be independent of the action of the thyroid hormone.

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OBSERVATIONS ON THE VOLUME OF DISTRIBUTION OF INULIN IN ANURIC SUBJECTS [1]

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Previous studies (1–5) on normal rats, dogs, and humans suggested that inulin was not metabolized, and that it provided an accurate and reproducible measure of some fixed volume of body fluid, presumably the extracellular fluid. This was established on the basis of : *(1)* virtually complete urinary recovery of inulin after infusion (1, 5); and *(2)* uniform distribution after 2 hours in dogs and 5 hours in man even though infusions were run for as long as 12 hours in the dog and 20.5 hours in man (1). In the absence of renal excretion, it was assumed that at equilibrium inulin would be distributed in a constant volume, and since it suffered no metabolic degradation it would afford a reasonably constant reference for changes in this volume. The present study was undertaken in an attempt to assess this premise.

METHODS

Purified inulin prepared by U. S. Standard Products Company was used. The various lots of inulin used were tested for fructose content by yeasting so that the actual amount of inulin injected could be accurately assessed. Inulin determinations on sera and urines were performed by Schreiner's modification of Roe's method (6), using venous sera in patients and arterial sera in dogs, and precipitating protein by the cadmium sulfate technique. Blood sugar determinations were done on all subjects, the highest noted being 191 mgm. per cent with the great majority under 120 mgm. per cent. In this laboratory, standard glucose solutions of 100 and 200 mgm. per cent develop color equivalent to 1.17 and 2.23 mgm. per cent of inulin respectively. Hence, yeasting was thought unnecessary.

Human studies

The 13 patients included in this study all had acute anuria of the "lower nephron nephrosis" type, with a variety of causes as listed in Table I. They were oliguric (urine volume less than 100 ml. per 24 hr.) from 2 to 10 days (average 7.4) before inulin was given. One intravenous injection of inulin was given, with the dose ranging from 4.2 to 13.6 gm. (average 7.3 gm.). The larger doses were administered later in the study in order that the higher serum levels obtained would minimize the effect of a rising serum inulinoid blank. In 2 patients, doses of inulin were also given 40 and 68 hours after the first dose.

All patients were on a regime of strict fluid restriction (500 to 800 ml. per day). During the 8 to 96 hours that serum inulin levels were followed, 7 patients lost an average of 1.1 kg. of body weight; 3 gained an average of 0.9 kg.; 2 had no change in weight; and 2 did not have second weighings.

In all cases, serum inulinoid blanks were determined prior to injecting inulin. Serial venous samples were obtained at varying intervals for 8 to 96 hours after injection. All urines were collected and analyzed for inulin; in 10 of 13 patients the volume of urine amounted to less than 100 ml. in the 24 hours after injection.

The apparent volume of distribution of inulin at any given time was calculated by dividing the amount administered (I), less the amount excreted in the urine (U),

TABLE I

Cause, duration and weight change of oliguria in human subjects

Pt.	Diagnosis	Days of oliguria†	Weight change in kg.**
E. B.	Sulfa, ? Shock Infectious Hepatitis	5	0
C. G.	Acute Yellow Atrophy	6	0
D. T.	$HgCl_2$ Intox. Prem. Sep. Placenta	8	+0.4
A. W.	Bilat. Cortical Necr.	8	−2.7
C. C.	Op. Shock—? Tx*	6	−1.0
R. A.	Tx*—Bleeding Ulcer	9	−0.8
C. I.	Tx*—Incompl. Abortion	8	−1.2
M. R.	Tx*—Postpartum	9	−0.2
A. S.	Meningitis—? Shock	9	?
D. B.	Idio. Myoglobinuria	7	+1.6
J. D.	? Induced Abortion, Intravasc. Hemolysis	5	0
E. B.	CCl_4 Intox.	10	+0.8
P. Mc.	Tx*—Postpartum	2	−0.6

* Tx = Transfusion Reaction
† Less than 100 ml. urine per day (before inulin)
** During Inulin Study

[1] This work was supported in part by grants from the Office of the Surgeon General, U. S. Army, under contract No. W-49-007-MD-497 and in part by grants from the National Heart Institute, U. S. Public Health Service, and from the American Heart Association.

[2] Established Investigator, American Heart Association.

by the serum inulin level (C) less the pre-injection blank (B) i.e., $V = \frac{I - U}{C - B}$. This volume then was expressed as a percentage of the body weight at time zero. Spaces were calculated at 8, 24, 48, 72, and 96 hours after injection, in cases in which it was possible to continue the study.

Inulin could not be detected in any significant amount in vomitus or feces, after preparing protein-free filtrates and performing determinations by the same methods as used for blood and urine. Urinary recovery of inulin during the post-oliguric diuresis was inaccurate because the amount excreted was masked by the high urinary inulinoid blank values.

Urinary inulinoid blank values were studied in several patients who did not receive inulin; these varied from 11 to 32 mgm. per cent.

Animal experiments

Studies were performed on 6 bilaterally nephrectomized dogs. Two were used to study the increase of inulinoid blank values alone as uremia progressed. In the other 4 dogs, which lived 7 or more days, a single intravenous dose of inulin was given 20 hours after nephrectomy; the dosage used was 0.2 gm. per kg. in 1 dog, 1.0 gm. per kg. in 3. These animals were given daily vitamins, antibiotics, and intravenous hypertonic glucose. They received nothing by mouth. Four dogs did not vomit until the fifth day or later; 2 others vomited less than 50 ml. daily before the fifth day. One animal had severe diarrhea. Fluid was restricted to permit an average weight loss of approximately 0.1 kg. daily. The initial weights of all dogs approximated 20 kg. Arterial serum samples were used for analysis; otherwise, spaces were determined and calculated as for the patients.

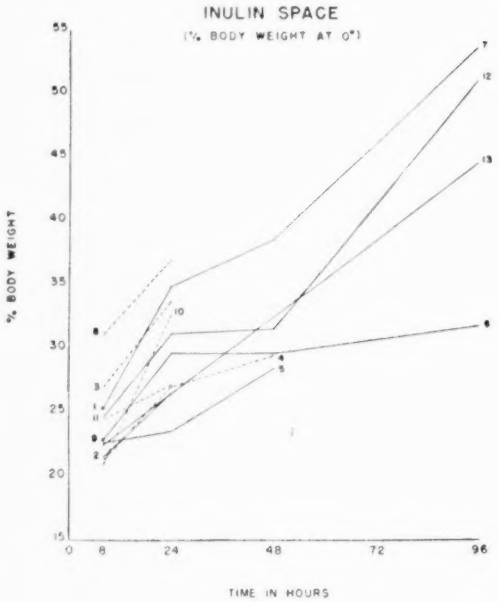

FIG. 1

RESULTS

The apparent volume of distribution of inulin ("inulin space") in patients at 8 hours averaged 24 per cent of body weight (20.8 to 31.0 per cent). Twenty-four hour spaces in 10 patients averaged

TABLE II

*Inulin spaces in anuric patients**

Pt.	Wt.	Inulin dose	8 Hr.		24 Hr.		48 Hr.		96 Hr.	
			Serum level	Space	Serum level	Space	Serum level	Space	Serum level	Space
	Kg.	Gm.	Mgm./100 ml.	% Wt.	Mgm./100 ml.	% Wt.	Mgm./100 ml.	% Wt.	Mgm./100 ml.	% Wt.
A. W.	96	4.4	21.6	21.2	17.0	27.0	15.6	29.4		
E. B.	71	4.4	24.5	25.3						
C. I.	64	4.2	25.3	25.3	17.0	34.9	13.7	38.6	7.0	53.6
C. C.	73	4.4	26.7	22.6	25.6	23.5	21.0	28.4		
C. G.	74	4.4	29.0	21.5	22.2	26.5				
D. T.	51.2	4.4	32.0	26.9	25.0	33.6				
R. A.	57.8	4.2	32.0	22.7	24.0	29.6	23.0	29.6	18.0	31.7
A. S.	85.0	7.3	37.8	22.7						
M. R.	58.4	9.0	49.8	31.0	41.7	37.0				
J. D.	73	9.7	54.4	24.4	48.2	27.1				
D. B.	84	9.9	56.8	20.8	36.2	32.6				
E. B.	58	9.7	68.0	24.6	52.3	30.7	48.5	31.5	26.0	51.0
P. Mc.	70.2	13.6	86.4	22.5					23.0	44.6
Average				24.0%		30.2%		31.5%		45.2%
Range				(20.8–31.0)		(23.5–37.0)		(28.4–38.6)		(31.7–53.6)

* Corrected for pre-injection inulinoid blank.

30.2 per cent (23.5 to 37 per cent). Spaces of 4 patients followed for 96 hours averaged 45.2 per cent (32 to 53.6 per cent). The data are shown in Table II and represented graphically in Figure 1.

In 2 of the above patients, repeat space determinations were made at 40 and 68 hours, by giving an additional dose of inulin, correcting for preinjection inulin level and calculating the space at 8 hours after this second injection. In one patient the first measurement was 16.8 liters and on repeating the determination with a second dose of inulin 40 hours later the space was 19.3 liters. In the second patient, the initial measurement was 16 liters and the second measurement 68 hours later was 17.5 liters. In the first patient the 40 hour "space" calculated from the disappearance curve of the initial inulin was 33 per cent and in the second patient a 68 hour space similarly calculated was 59 per cent.

In an effort to determine the effect of anuria on the serum inulinoid blank, 2 bilaterally nephrectomized dogs were studied for 117 and 164 hours respectively without giving inulin. In each case, there was a gradual and progressive rise in the serum blank values from an initial value of 1.7 and 1.5 mgm. per cent to 7 mgm. per cent at 117 hours and 11.5 mgm. per cent at 164 hours.

One bilaterally nephrectomized dog was given inulin in an amount similar to that given patients (0.2 gm. per kg.). At 2 hours, the serum inulin level, corrected for zero hour blank value, was 95 mgm. per cent with an inulin space of 17 per cent of body weight. At 120 hours, the actual serum level was 16 mgm. per cent, but if corrected for the serum blank at 120 hours, as determined in the contrast study, the true serum inulin level approached the serum inulinoid blank value.

To minimize the effect of varying serum blanks, 3 bilaterally nephrectomized dogs were given large doses of inulin (1.0 gm. per kg.). Initial serum levels ranged from 530 to 600 mgm. per cent and the 2 hour spaces were 18, 18.4 and 19 per cent of body weight. In each instance, there was a gradual and progressive decline in the serum levels, averaging 0.5 per cent of the administered dose per hour.

One animal died at 95 hours with a space of 31 per cent. A second dog's space was 40.5 per cent

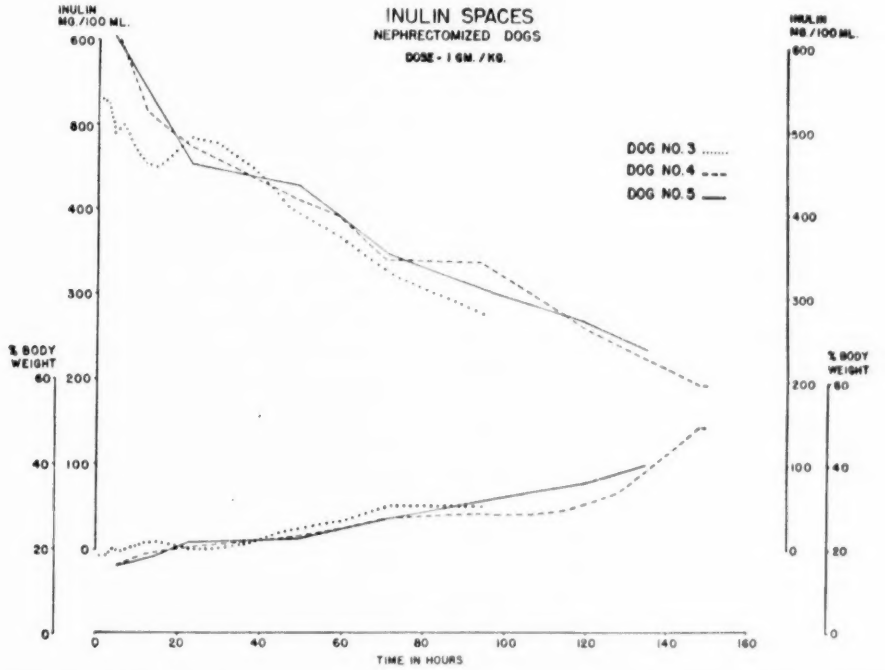

FIG. 2

at 133 hours and the third showed an "inulin space" of 50 per cent at 150 hours. The results are shown in Figure 2. It is interesting to note that the values for serum levels and inulin spaces are virtually identical in all 3 animals, particularly since one dog had severe diarrhea and the others did not. In this case, loss was carefully measured and replaced by intravenous fluids.

In order to exclude the possibility of inhibiting substances in uremic serum, known amounts of inulin were added to the serum of both humans and dogs and allowed to incubate at body temperature under sterile conditions for varying periods up to 24 hours. Recovery was 98 to 101 per cent in all tests.

In addition, sera from 4 patients, who received inulin, were analyzed before and after 6 hour runs on the Kolff artificial kidney. In a typical experiment blood urea nitrogen concentration was decreased from values of 313 mgm. per 100 ml. to 65 mgm. per 100 ml. with comparable changes in other retained metabolites. Inulin values in the 4 patients decreased 2 to 4.5 mgm. per cent. Three patients who had not received inulin were studied in a similar manner. Initial inulinoid blanks varied 2.5 to 4.9 mgm. per cent. Values following dialysis were reduced by 2 to 4 mgm. per cent. Thus, it appears that the inulinoid blank diffuses through a cellophane membrane whereas inulin does not.

DISCUSSION

The data presented indicate that inulin does not equilibrate in any fixed volume of body fluid in anuric patients as shown by the steady and continuous fall of inulin levels. That this decrease in inulin concentration could not be accounted for by urinary or other losses or by interference with the determination is suggested by the following:

(*1*) *Total urinary inulin content was small during space studies and urinary inulinoid blank values were high.*

(*2*) *Inulin could not be detected in significant amounts in the gastrointestinal contents.* The possibility of degradation of inulin in the gastrointestinal tract prior to analysis must be considered, but evidence from experiments on dogs does not support this supposition, since the 3 animals receiving large doses of inulin show practically superimposable curves for both serum inulin concentrations and "inulin spaces." Of these, 2 animals had minimal gastrointestinal losses, while one had severe diarrhea throughout the study, replaced volume for volume by saline. This argues against loss of inulin into the gastrointestinal tract.

(*3*) *Incubation of inulin with uremic (anuric) sera at body temperature for as long as 24 hours did not prevent complete recovery of inulin.* Thus, it seems unlikely that interfering substances invalidate the determinations.

The serum inulinoid blank is high in uremia and rises in anuric dogs from initial concentrations of 1 to 3 mgm. per cent to as high as 12.5 mgm. per cent in our studies. This change is variable in different subjects and cannot be correlated with blood urea levels or other indices of uremia. Glucose contributes to this blank about 1 mgm. per cent per 100 mgm. per cent glucose. In our series, the glucose levels did not vary greatly and did not become high enough to account for the high blank values observed. The cause of this high inulinoid blank has not been determined. Our observations on patients undergoing hemodialysis indicate that the artificial kidney will remove some substance or substances present in the blood responsible for the elevated blank values in uremia.

When smaller doses of inulin are given (0.2 gm. per kg.), the blank factor becomes very significant after 3 to 4 days of anuria. In the experiments on dogs if the serum inulin levels of one animal are corrected for the blank value observed in another, serum levels approach the blank value between 100 to 160 hours. Therefore, virtually all inulin has disappeared from the serum, indicating an inulin space of nearly 100 per cent of body weight. If large amounts of inulin are administered (1 gm. per kg.), the blank factor becomes insignificant.

The observations relating to this subject made by other workers may be summarized as follows:

(*1*) Gaudino and Levitt (*2*) studied 3 bilaterally nephrectomized dogs utilizing a single injection of inulin. They stated that a uniform distribution was obtained in 1 to 2 hours, a period similar to that observed in normal dogs. However, it was noted that the inulin space began to increase after 6 hours. This was attributed to a terminal pathological shift of intracellular water.

(*2*) Kruhøffer (*3*) studied 13 nephrectomized rabbits and one nephrectomized dog. In the former, equilibration of inulin had not occurred when followed as long as 10 hours. In the latter,

the inulin space was still expanding at 12 hours.

(*3*) Berger and his coworkers (*7*) measured inulin spaces in 6 nephrectomized dogs. In all animals, a slow and continuous fall in inulin plasma levels was noted, varying from 0.15 to 0.72 per cent per hour (our animals averaged 0.5 per cent per hour). They attributed this to either slow metabolism or changing cellular permeability.

(*4*) Cotlove (8) analyzed inulin and chloride in rat muscle after infusions of 1, 2, 6 and 15 hours. The inulin space showed a progressive approximation to the chloride space with increasing time of infusion.

(*5*) Nichols and his associates (9) studied 8 dogs after a single injection of inulin. Inulin spaces in muscle showed a continuous increase from 3 to 6 hours. Studies were not carried beyond this period.

Among possible explanations for the observed data, the following are to be considered: (*a*) a metabolic degradation of inulin, (*b*) a valid expansion of the extracellular fluid space during anuria, and (*c*) a shift of inulin into cell, bone, or connective tissue or other body water not included in the "extracellular fluid" as defined in 6 to 8 hour "inulin space" in normal subjects.

A real expansion of extracellular fluid probably does occur in many anuric patients who are overhydrated, but this cannot explain changes of the magnitude observed. The calculated "inulin spaces" in patients reached values as high as 40 to 50 per cent of body weight without clinical evidence of over-hydration. Furthermore, in dogs, which could be followed longer, the "inulin spaces" reached the impossible value of nearly 100 per cent of body weight.

The other possibilities are more difficult to exclude, and additional studies are necessary to determine which is correct. Nichols and associates (9) observed that the inulin space determined by tissue analysis of muscle and connective tissue of anuric animals, gradually increases after an injection of inulin. However, this would not explain the magnitude of change we observed in dogs given large doses of inulin. Because of this, it is probable that destruction of inulin does occur either in the extracellular fluid or after penetration into cells or both. If so, it is possible that there is a similar, slow degradation of inulin in non-anuric subjects which cannot be detected by the methods now employed to measure inulin space. Such a loss is apparent in the data of Gaudino and Levitt (*2*) although they attribute it to redistribution of fluid.

SUMMARY AND CONCLUSIONS

1. Thirteen patients with acute anuria were studied using a single injection of inulin. A steady and continuous fall of the serum inulin levels and rise in "inulin space" values was observed.

2. A similar phenomenon was noted in bilaterally nephrectomized dogs and could not be explained by changing blank, external losses, dilution or interfering substances.

3. The volume of distribution of inulin as calculated from serum levels after a single dose in the anuric subject shows a progressive increase, the magnitude of which probably indicates a disappearance of the injected inulin rather than a valid increase in "inulin space."

ACKNOWLEDGMENTS

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THE VALUE OF NEEDLE BIOPSY IN THE CHEMICAL ESTIMATION OF LIVER LIPIDS IN MAN[1]

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INTRODUCTION

Specimens of liver tissue obtained by needle biopsy may be subjected to various forms of study. Our interest in the fatty liver has led to the development of micro-chemical methods based on standard techniques for the quantitative analysis of lipids in the liver specimens in order to supplement the histological information obtained. The value of such determinations is dependent on the extent to which the specimen of liver obtained by the needle method is representative of the liver as a whole in respect to lipids. Some workers have questioned the validity of the lipid values obtained on analysis of a single liver specimen and have felt that particularly in cirrhosis marked variation in lipid content can occur. Waldstein and Szanto (1) using the needle technique have, however, reported that histological examination of specimens obtained at necropsy from 9 patients with cirrhosis, 2 cases of toxic hepatitis and 4 "normal" patients, indicates that fatty metamorphosis is uniform throughout the human liver. Rourke and Stewart (2), and Theis (3) have also shown by chemical analysis of 1 gram samples taken from different sites in the liver that the distribution of lipids is relatively uniform.

In the present study, specimens of liver have been taken from 18 different sites in each of 25 livers and examined chemically and histologically for lipids. The variation in lipid content in the different specimens is reported and the influence of cirrhosis on the interpretation of the findings is discussed.

TECHNIQUES

Material

The livers used in this study were obtained from patients at the Cincinnati General Hospital who had died from a variety of diseases (Table I). In the selection of material preference was given to livers showing gross evidence of cirrhosis and/or fatty vacuolization, so that of the 25 livers examined there were 13 non-fatty livers (Group I), 5 of which were cirrhotic, and 12 fatty livers (Group II), 4 of which were cirrhotic. Necropsies were performed within 6 hours of death, and the liver was then removed for immediate analysis. Ralli and her associates (4) have shown that in dogs autolysis during the first 6 hours after death does not cause appreciable change in the lipid content of the liver.

Using the Vim-Silverman needle, specimens of liver tissue were obtained from approximately 18 different sites as indicated in Fig. 1. Position 5 was that from which biopsy samples are usually obtained in living subjects using the intercostal approach. The sample was immediately removed from the needle with forceps, dried with gauze to remove adherent blood and cut into two; one half used for histological examination and the other, weighing 5 to 8 mg., for chemical analysis.

Weight measurements

In the present work all weighings were made using a single beam quartz fibre balance as described by Schmidt-Nielsen and Taylor (5). This was constructed to take a maximum load of 12 mg. Calibration showed that there was a linear relationship between the dial reading and the load and that 280 scale divisions were equivalent to a load of 1 mg. An error of less than 1 per cent was ob-

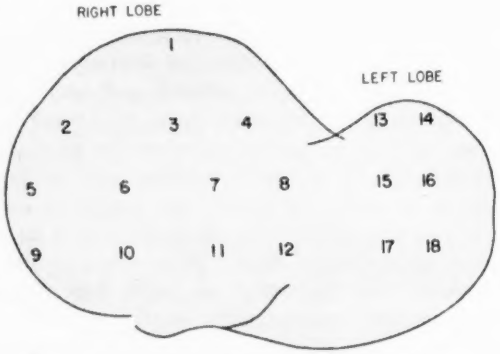

FIG. 1. POSITIONS IN LIVER FROM WHICH NEEDLE SPECIMENS WERE TAKEN

[1] Aided by grants from Samuel Freedman, the Robert Gould Research Foundation, the Milton Brown Memorial Fund, and the John Eisman Memorial Fund.

served for samples weighing 1 mg. whereas with larger samples the error was even smaller. Reproducibility of weighings was found to be excellent, and the speed at which these could be carried out proved advantageous.

Wet weight: In the course of weighing specimens of liver tissue obtained by the needle method there is considerable evaporation of water. Schmidt-Nielsen and Taylor (5) showed that the rate of water loss from a section of fresh rat liver is constant for several minutes after it is cut. They obtained the wet weight of the fragment by making repeated weighings and then extrapolating back to zero time. The same method was found to be applicable to the determination of the wet weight of liver specimens obtained by the needle method.

As soon as the needle was opened for the removal of the specimen a stop watch was started and the portion to be used for chemical analysis transferred to a glass hook of known weight (approximately 1 mg.). The hook was then placed on the balance and weighings were made at 15 second intervals for the next 2 minutes. The results were plotted on graph paper and the straight line obtained was extrapolated back to zero time to give the wet weight (Fig. 2).

Dry weight: The liver specimen was frozen at $-24°C$ and then dried in vacuum over anhydrous calcium sulphate for 48 hours after which there was no further loss in weight. When dry, the specimen adhered to the glass hook, which could then be used as a carrier in all subsequent operations. Repeated drying in an oven at $105°C$ did give further small losses in weight, attributable to the loss of volatile substances other than free water. It is not possible to obtain exactly comparable results using the two methods for obtaining dry weights. The vacuum drying technique, however, has the advantage that oxidation of the lipid matter is prevented.

Total lipid content: Directly after weighing, the hooks

TABLE I

Summary of clinical and histological data

Group	Patient	Cause of death	Weight of liver g.	Histological classification of liver	Histological estimation of fat	Total lipids Mean ± S.D. g./100 g. liver
I(a)	H. B.	Carcinoma prostate	950	N.D.A.R.†	Absent	5.0 ± 0.8
	W. T.	Lobular pneumonia	1050	N.D.A.R.	Absent	5.1 ± 0.8
	M. D.	Subdural hematoma	1500	N.D.A.R.	Slight	6.5 ± 0.8
	G. S.	Gunshot wound	—	N.D.A.R.	Slight	6.3 ± 0.5
	P. H.	Lobular pneumonia	1050	Acute passive congestion	Absent	5.2 ± 0.4
	A. W.	Carcinoma prostate	1005	Acute passive congestion	Slight	6.1 ± 0.5*
	F. R.	Myocardial infarction	1205	N.D.A.R.	Absent	4.3 ± 0.3*
	F. C.	Gunshot wound	1525	Chronic nonspecific hepatitis	Slight	6.9 ± 0.6
I(b)	D. W.	Lung abscess	1600	Portal cirrhosis	Slight	7.0 ± 0.9
	C. H.	Myocardial infarction	1275	Portal cirrhosis	Slight	5.0 ± 0.7
	P. T.	Cerebral softening	1660	Portal cirrhosis	Absent	4.8 ± 0.6
	G. S.	Peritonitis	1875	Portal cirrhosis	Slight	4.3 ± 1.0
	H. D.	Perforated peptic ulcer	—	Portal cirrhosis	Absent	6.2 ± 0.5
II(a)	R. R.	Cerebral thrombosis	2520	Acute passive congestion	Moderate	13.8‡
	J. R.	Myocardial infarction	1975	Fatty vacuolization	Severe	20.3 ± 1.7
	W. D.	Lobar pneumonia	1500	Fatty vacuolization	Moderate	9.4 ± 0.7
	W. G.	Lobar pneumonia	1630	N.D.A.R.	Slight	8.9 ± 0.7
	E. C.	Intracerebral hemorrhage	2200	Severe fatty vacuolization	Severe	33.5 ± 1.8*
	E. N.	Acute liver necrosis	2355	Fatty vacuolization. Massive necrosis	Moderate	12.9 ± 1.3*
	J. B.	Diabetic coma	2000	Fatty vacuolization	Moderate	9.5 ± 0.7
	C. C.	Lobar pneumonia	—	Severe fatty vacuolization	Severe	29.0 ± 2.9
II(b)	V. M.	Portal cirrhosis	3175	Portal cirrhosis	Severe	28.3 ± 4.1
	J. Y.	Portal cirrhosis	2000	Portal cirrhosis	Severe	20.0 ± 3.5
	M. T.	Portal cirrhosis	4080	Portal cirrhosis	Severe	30.1 ± 2.9
	G. T.	Septicemia	3900	Portal cirrhosis	Moderate	14.9 ± 2.2

* Determined on dry tissue only.
† N.D.A.R.: No diagnostic abnormalities recognized.
‡ Insufficient chemical analyses for statistical analysis and inclusion in Fig. 6.

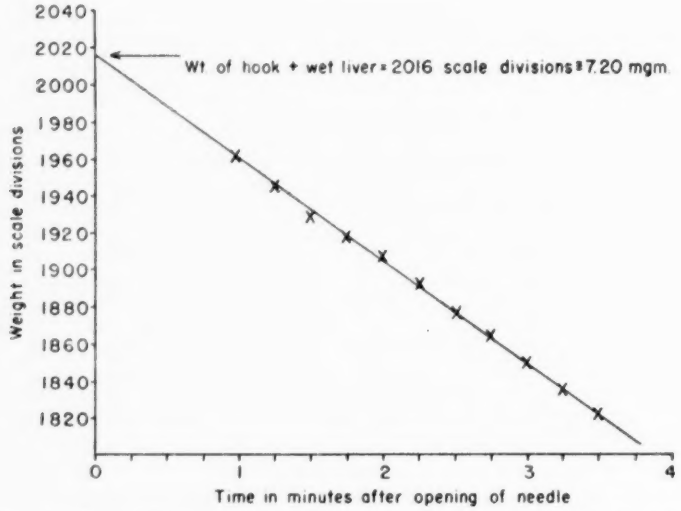

FIG. 2. DETERMINATION OF WET WEIGHT OF NEEDLE SPECIMEN OF LIVER BY MEASUREMENT OF THE RATE OF LOSS OF WATER

with the dried liver specimens were placed in glass tubes (80 mm. long and 4 mm. diameter), sealed at one end. Five hundred μl. ethyl alcohol (or Bloor's Reagent, containing 3 volumes alcohol to 1 volume ethyl ether) were added and the open end of the tube was sealed. The glass vials were then kept in an incubator for 48 hours at 60°C and shaken at intervals. After cooling, aliquots of the solvent were used for lipid analysis. The liver specimens were dried and reweighed and the difference between this weight and that of the dried tissue was taken to be the total lipid content.

Preliminary experiments showed that further extraction with fresh alcohol produced no appreciable change in the weight of the lipid free residue even with the very fatty livers, and that under the above conditions ethyl alcohol and Bloor's Reagent were equally satisfactory extracting agents. In several instances 10 g. specimens of liver from which needle specimens had been taken were dried and extracted with ethyl alcohol in a Soxhlet apparatus and the results obtained compared favorably with those of the needle specimens. The macro-method in these cases gave results of 16.5, 19.4, 16.1, 35.8, 11.8, 15.8 and 11.4 g. per 100 g. dry liver respectively as compared with 15.6, 19.0, 15.3, 36.0, 10.8, 15.9 and 11.4 g. by the micro-method.

Chemical analysis of lipids

Constriction pipettes were used for all volumetric measurements made in the following determinations (Linderstrøm-Lang and Holter [6]).

Phospholipids: Three hundred μl. of the alcoholic extract were used for the determination of lipid phosphorus. The alcohol was removed by evaporation in a boiling water bath and 500 μl. 5N H_2SO_4 were added. The samples were heated at 90°C for 2 hours and then at 160°C until charring had occurred. Two hundred and fifty μl. 30 per cent hydrogen peroxide (superoxol) were added to the samples, the blank (500 μl. 5N H_2SO_4) and the standard (prepared in 500 μl. 5N H_2SO_4) and all were reheated for an hour at 160°C after charring had disappeared. When necessary more H_2O_2 was added to all the tubes, which were reheated for 1 hour at 160°C to remove excess H_2O_2. After cooling 3 ml. water, 1 ml. 10 per cent sodium molybdate and 1 ml. 0.5 per cent p-methyl aminophenol sulphate in 3 per cent sodium bisulphite were added and in 15 minutes the color developed was read using a Beckman Spectrophotometer at 660 mμ. The lipid phosphorus content was multiplied by 25 to give the value for phospholipid.

Total fatty acids and iodine number: The total fatty acids and their iodine number were determined on 140 μl. of the alcoholic exract using the micro-methods described by Schmidt-Nielsen (7–10). The extract was pipetted into a vial of thin glass (60 mm. × 3 mm.) and the alcohol immediately removed in a vacuum desiccator. Twenty μl. 50 per cent alcoholic KOH were used to saponify the lipids in the presence of 60 μl. toluene. The tube was sealed and placed in a water bath at 80°C for 30 minutes. After cooling, the vial was centrifuged for 1 minute and the alcohol and toluene removed in a vacuum desiccator containing paraffin shavings. Fifty-seven μl. N/2 HCl were added to acidify the mixture and 60 μl. toluene were pipetted for the extraction of the fatty acids. The tube was immediately sealed and shaken 30 to 50 times to obtain complete extraction. After centrifugation for 1 minute, aliquots of the toluene layer were taken for titration of the fatty acids and iodine number. The quantities of toluene extract used were adjusted so

that for the fatty acid determination not more than 100 μg. of fat was present, while the maximum amount possible was available for the iodine number determination. The latter was found to give reproducible results when there were 30 μg. fatty acids or more present; with smaller quantities the difference between the blank and the titration reading was too small to give accurate results. A solution of 284.5 mg. stearic acid per 100 ml. toluene was used for the standard and all readings for the fatty acids were expressed in terms of the equivalent amount of stearic acid.

Histological techniques

The portion of tissue taken for histological examination was fixed in 10 per cent formalin prepared with 2 per cent calcium acetate. Part of the specimen was sectioned in paraffin and stained with hematoxylin-eosin. Frozen sections were made of the remainder; these were stained with Sudan IV, Oil-Red O and Sudan Black.

Each section was graded for visible fat using the designations of "absent," "slight," "moderate" or "severe." If no vacuolization could be found, with hematoxylin-eosin staining, the designation of "absent" was used, even though these sections might be faintly positive for lipid with fat stains. When only spotty fatty vacuolization was noted, the section was graded as "slight." The fat infiltration was regarded as "moderate" when not more than one third of the cells contained fat. Sections in which the fatty vacuolization was greater than this were given the classification of "severe."

RESULTS

A liver has been designated "fatty" if the lipid content is greater than 8 g. per 100 g. liver, or if the fatty vacuolization was classified as "moderate" or "severe."

Percentage of dry matter

The amount of dry matter was determined in each of the specimens examined. In non-fatty livers the mean values ranged between 22 and 33 per cent with an average of 27.9 per cent (*i.e.*, 72.1 per cent water content). In fatty livers a rough linear relationship was found between the percentage of dry matter and the amount of lipid in 100 g. dried tissue; this finding confirms the observations of other workers using macro-techniques (Eger [11] and Halliday [12]) (Fig. 3). This relationship was not apparent in non-fatty livers.

Total lipids

The results obtained for the total lipids in 13 non-fatty livers and 12 fatty livers have been expressed as g. lipid per 100 g. wet liver and have been recorded in Figs. 4, 5, 6, 7 to show the varia-

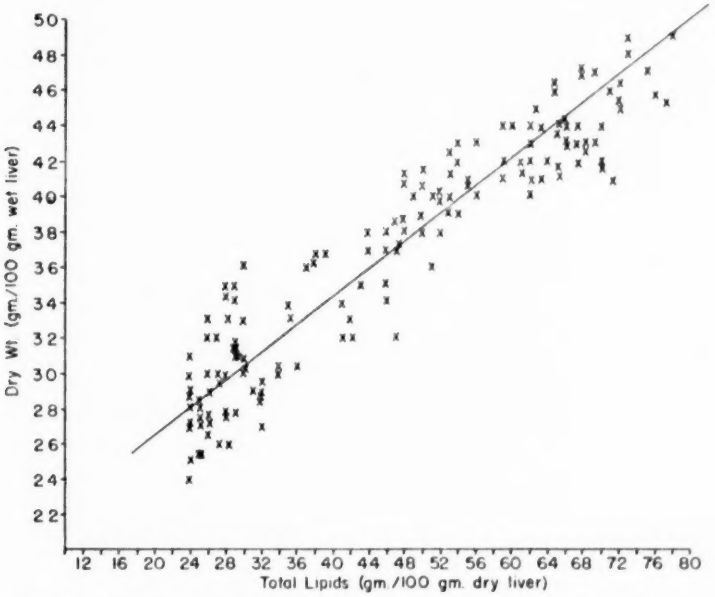

FIG. 3. THE RELATIONSHIP BETWEEN THE NUMBER OF GRAMS OF DRY MATTER PER 100 G. WET LIVER AND THE NUMBER OF GRAMS OF TOTAL LIPIDS PER 100 G. DRY LIVER

TABLE II

Variations in the dry matter, lipids and visible fat in needle specimens from a "normal" liver (F. C.). Group I(a)—Table I

Specimen No.	Dry matter g./100 g. wet liver	Total lipids g./100 g. wet liver	Total fatty acids g./100 g. wet liver	Phospho-lipids g./100 g. wet liver	Histological estimation of fat
1	31.6	6.4	—	2.6	slight
2	33.4	6.6	4.9	2.7	slight
3	33.9	7.1	4.8	2.9	slight
4	34.0	7.2	5.0	2.7	slight
5	33.3	7.8	5.2	2.9	absent
6	33.7	7.2	4.9	2.9	absent
7	33.2	6.0	4.5	2.7	absent
8	33.0	7.1	4.8	2.8	absent
9	34.7	6.2	4.2	2.8	absent
10	32.9	6.1	4.4	—	absent
11	32.5	7.5	4.9	2.8	slight
12	33.9	6.8	4.5	2.8	slight
13	33.3	6.9	4.8	2.9	slight
14	34.5	7.8	5.1	3.2	slight
15	31.4	6.2	4.6	—	slight
16	33.4	7.0	4.7	2.9	slight
17	33.0	7.4	5.0	3.0	slight
18	34.5	7.3	5.3	3.3	slight

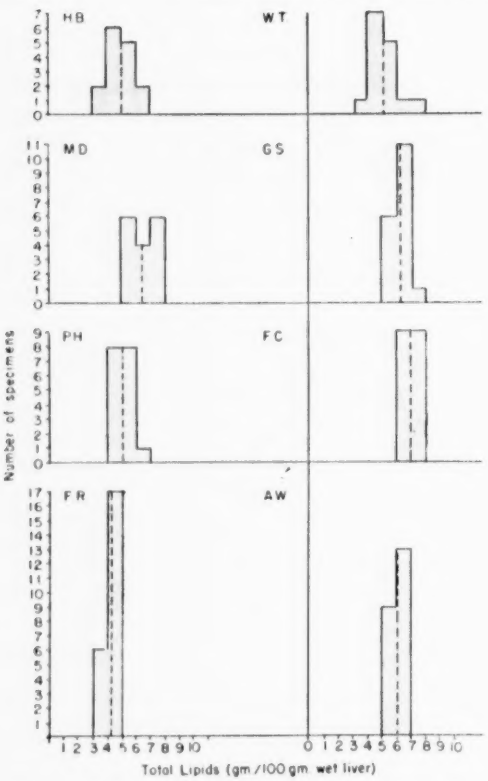

FIG. 4. GROUP I (A). THE DISTRIBUTION OF THE TOTAL LIPID CONCENTRATIONS IN SPECIMENS TAKEN FROM NON-FATTY, NON-CIRRHOTIC LIVERS

In this and subsequent figures the interrupted vertical line indicates the mean value for the analyses.

tion found in the different specimens from each liver. Tables II, III, IV, V give the detailed results obtained in a case representative of each group studied. In neither the non-fatty nor fatty livers was there a consistent difference between the lipid concentrations in the right or left lobes of the liver.

In 4 livers (marked with an asterisk—Table I) the lipid concentration of the specimens was determined in dry tissue only and approximate wet weights were calculated using Fig. 3 so that the results could be recorded in g. per 100 g. wet liver.

Non-Fatty Livers: Non-Cirrhotic (Fig. 4, Table II): Eight livers with no histologic evidence of intrinsic disease were examined. Values ranging from 3.5 to 7.8 g. lipid per 100 g. wet liver for individual specimens were recorded giving a mean value of 5.7 g. lipid per 100 g. wet liver for the group.

Cirrhotic (Fig. 5, Table III): Five of the livers studied were found on histological examination to be cirrhotic and to have only minimal fatty vacuolization. Values for individual specimens ranged from 2.3 to 8.7 g. lipid per 100 g. wet liver giving a mean value for the group of 5.5 g. per 100 g. wet liver. The specimens tended to show greater variance in their lipid content than was found in the non-cirrhotic group; this variance did not appear to be directly related to the amount of fibrosis present.

Fatty Livers: Non-Cirrhotic (Fig. 6, Table IV):

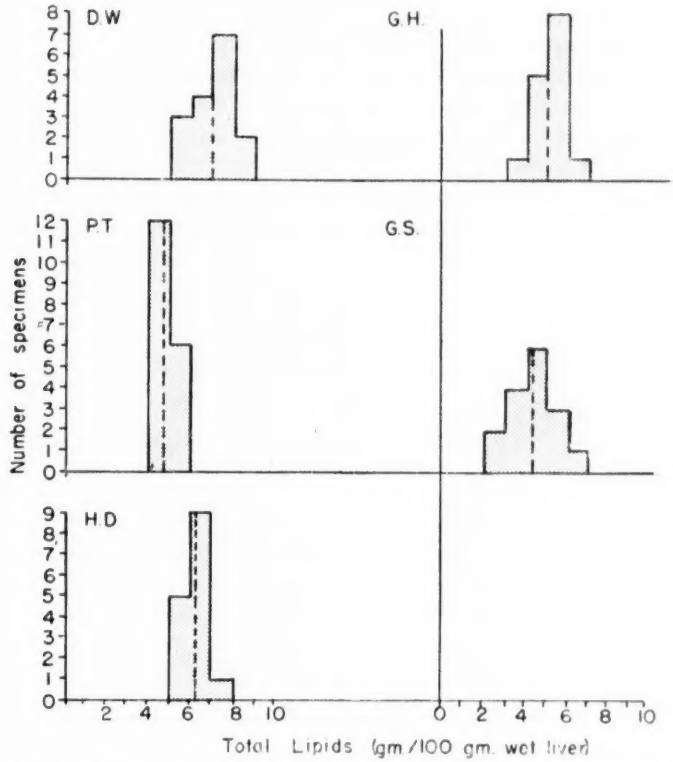

FIG. 5. GROUP I (B). THE DISTRIBUTION OF THE TOTAL LIPID CONCENTRATIONS IN SPECIMENS TAKEN FROM NON-FATTY, CIRRHOTIC LIVERS.

TABLE III

Variations in the dry matter, lipids and visible fat in needle specimens from a non-fatty, cirrhotic liver (P. T.). Group I (b)—Table I

Specimen No.	Dry matter *g./100 g. wet liver*	Total lipids *g./100 g. wet liver*	Total fatty acids *g./100 g. wet liver*	Iodine No. of fatty acids	Phospho-lipids *g./100 g. wet liver*	Histological estimation of fat
1	22.8	5.3	2.3	135	2.4	absent
2	22.0	4.0	2.0	137	2.1	absent
3	21.9	4.4	2.7	—	2.3	absent
4	23.1	5.5	2.2	134	2.1	absent
5	23.4	4.5	2.1	137	2.0	absent
6	22.7	5.5	1.9	130	2.2	absent
7	23.0	4.3	2.2	—	2.3	absent
8	22.8	4.0	2.1	135	2.2	absent
9	21.7	4.0	1.9	—	2.2	absent
10	22.2	4.0	1.8	—	2.0	absent
11	22.3	4.8	2.0	133	2.1	absent
12	23.0	5.2	2.2	136	2.4	absent
13	23.9	5.6	2.2	139	2.4	absent
14	21.7	4.7	2.0	132	2.1	absent
15	23.0	4.7	2.1	138	2.2	absent
16	21.5	4.8	1.9	132	2.1	absent
17	21.7	4.8	2.0	138	2.0	absent
18	22.3	5.5	2.5	131	2.3	absent

This group consisted of 8 livers, mean values for the lipid content of which ranged from 8.9 to 33.5 g. per 100 g. wet liver (group mean 17.6 g. per 100 g.). The absolute variations between the lipid content of the specimens in this group were greater than those observed in the non-fatty liver groups and tended to increase as the lipid content of the liver increased. The variance per gram of lipid was, however, less than that found in the other groups.

Cirrhotic (Fig. 7, Table V): In 4 of the fatty livers, different degrees of fibrosis were encountered and there was considerable variation in the values for the lipid content of the specimens in a particular liver. This variation exceeded that found in the previous groups and was roughly pro-

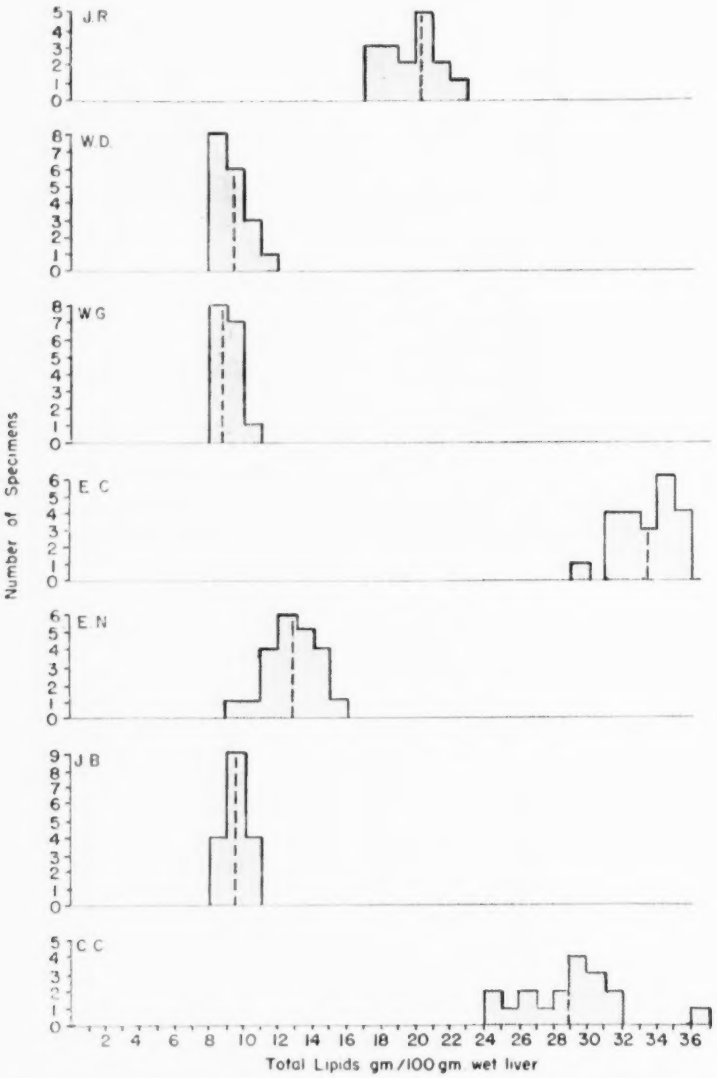

FIG. 6. GROUP II (A). THE DISTRIBUTION IN THE TOTAL LIPID CONCENTRATIONS IN SPECIMENS TAKEN FROM FATTY, NON-CIRRHOTIC LIVERS

TABLE IV

Variations in the dry matter, lipids and visible fat in needle specimens from a fatty, non-cirrhotic liver (J. R.). Group II(a)—Table I

Specimen No.	Dry matter *g./100 g. wet liver*	Total lipids *g./100 g. wet liver*	Total fatty acids *g./100 g. wet liver*	Iodine No. of fatty acids	Phospho-lipids *g./100 g. wet liver*	Histological estimation of fat
1	36.8	17.5	—	—	3.4	severe
2	38.8	18.3	15.3	64	—	severe
3	38.8	17.9	14.9	—	3.0	severe
4	39.4	19.0	15.7	61	3.0	severe
5	40.7	19.8	—	—	3.0	severe
6	40.9	21.5	18.7	63	3.0	severe
7	41.8	21.2	—	—	2.9	severe
8	40.8	21.9	14.0	64	3.2	severe
9	38.4	17.5	14.5	65	—	severe
10	38.3	18.7	14.8	68	2.9	severe
11	40.3	22.0	18.2	67	3.0	severe
12	40.9	20.0	16.7	65	—	severe
13	40.4	22.2	16.3	67	—	severe
14	42.0	20.7	17.6	64	—	severe
15	40.2	21.3	17.7	64	3.2	severe
16	41.5	21.5	18.9	66	—	severe
17	38.7	20.5	—	—	—	severe
18	42.9	23.5	18.0	68	3.0	severe

portional to the lipid content of the liver. Mean values for the lipid content in this group ranged from 14.8 to 30.1 g. per 100 g. liver (group mean 23.3 g. per 100 g.).

Total fatty acids

As seen in Tables II to V, the values for the total fatty acids generally paralleled those for the total lipids. Variations in total lipids for individual specimens particularly in the fatty livers appear, therefore, to be due mainly to variations in the neutral fat content.

Iodine number

Determination of the iodine number of the fatty acids present in the different specimens gave consistent findings for any particular liver. Tables V and VI show that this constancy is maintained

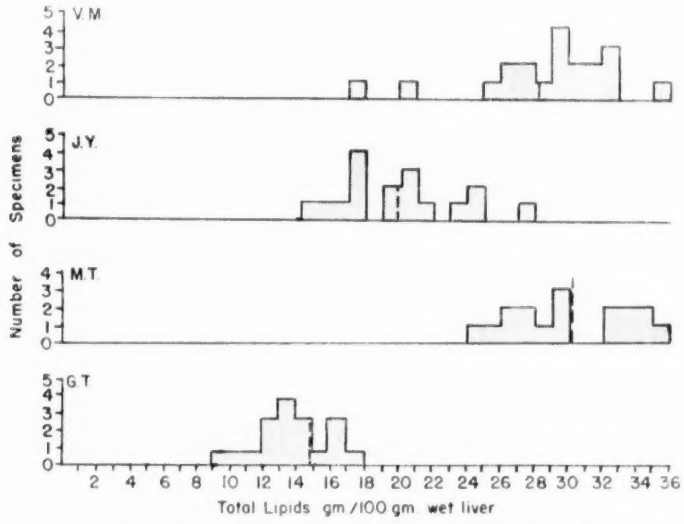

FIG. 7. GROUP II (B). THE DISTRIBUTION IN THE TOTAL LIPID CONCENTRATIONS IN SPECIMENS TAKEN FROM FATTY, CIRRHOTIC LIVERS

TABLE V

Variations in the dry matter, lipids and visible fat in needle specimens from a fatty, cirrhotic liver (M. T.). Group II(b)—Table I

Specimen No.	Dry matter g./100 g. wet liver	Total lipids g./100 g. wet liver	Total fatty acids g./100 g. wet liver	Iodine No. of fatty acids	Phospho-lipids g./100 g. wet liver	Histological estimation of fat
1	47.2	34.4	29.9	—	2.2	severe
2	44.2	27.9	24.1	—	2.4	severe
3	42.7	26.3	23.2	70	2.1	severe
4	—	—	—	—	—	severe
5	45.1	34.9	29.5	70	2.3	severe
6	41.2	26.2	25.3	68	2.1	severe
7	41.1	25.8	23.2	67	2.1	severe
8	43.5	29.0	—	—	2.1	severe
9	45.3	32.7	28.1	66	1.7	severe
10	43.4	27.0	25.7	66	2.2	severe
11	46.6	35.9	29.3	67	2.1	severe
12	46.7	33.8	30.6	67	1.9	severe
13	43.5	28.4	25.4	67	2.2	severe
14	41.2	24.5	20.8	69	2.3	severe
15	44.6	29.5	23.0	74	2.1	severe
16	43.3	29.6	27.3	67	2.1	severe
17	45.0	32.5	28.5	68	1.8	severe
18	47.0	33.4	29.0	67	2.1	severe

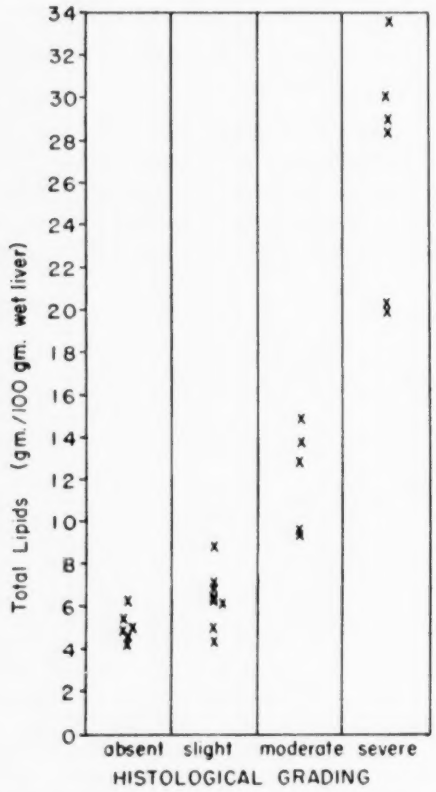

FIG. 8. THE RELATIONSHIP BETWEEN HISTOLOGICAL GRADING AND THE CHEMICAL DETERMINATION OF TOTAL LIPIDS (MEAN VALUE FOR LIVER)

even when the total lipid content shows some variation.

Phospholipids

Phospholipids appeared to be fairly uniformly distributed throughout both non-fatty and fatty livers. Slightly lower values were obtained for cirrhotic than for non-cirrhotic livers. The values recorded tended to be somewhat higher than those reported by other workers (4) possibly due to the method of extraction, which did not involve the use of petroleum ether.

Histological examination

Individual grading of the sections revealed relative uniformity in the amount of fat in the various

TABLE VI

Limits of error in the prediction of the lipid content of the whole liver from a single biopsy specimen and minimum change in lipid content of specimen indicative of a significant decrease of lipid content of whole liver upon repeat biopsy

Classification of liver	Approximate limits	Minimum differences indicative of significant decreases	
		Single sample	Paired samples
Non-fatty, non-cirrhotic	x ± 25%	0.39x	0.22y
Non-fatty, cirrhotic	x ± 35%	0.50x	0.30y
Fatty, non-cirrhotic	x ± 18%	0.31x	0.17y
Fatty, cirrhotic	x ± 30%	0.47x	0.28y

x = lipid content of specimen in g./100 g. liver.
y = mean lipid content of control pairs of samples in g./100 g. liver.

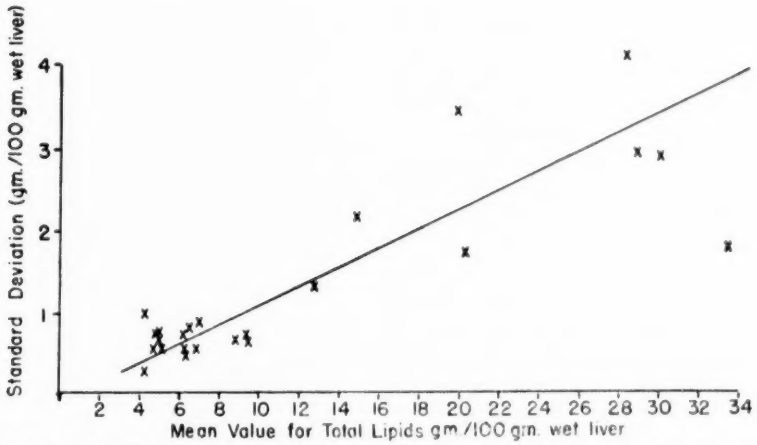
Standard Deviation (gm./100 gm. wet liver)
4
3
2
1
2 4 6 8 10 12 14 16 18 20 22 24 26 28 30 32 34
Mean Value for Total Lipids gm./100 gm. wet liver

FIG. 9. THE RELATIONSHIP BETWEEN THE VARIANCE IN THE SPECIMENS AND THE MEAN TOTAL LIPID CONCENTRATION

portions of the liver. The impression gained of the amount of fat present by study of the hematoxylin-eosin stained sections, supplemented by the various fat stains, showed good correlation with the amount of fat found by quantitative methods (Fig. 8). With one exception (W. G.) livers graded for lipids as "absent" or "slight" had lipid contents of less than 8 g. lipid per 100 g. wet liver. Those graded "moderate" tended to have values ranging between 8 and 16 g. lipid per 100 g. wet liver, while those with greater amounts of lipid were all classified as "severe."

DISCUSSION

The present work has been designed to determine the possibility of estimating the lipid concentration of the entire liver from specimens obtained by needle biopsy. The error of this estimate will depend upon the accuracy of the chemical analysis and the distribution of lipids in the human liver, in various pathological conditions.

That there is no important systematic deviation between the analysis made by the micro-methods, described in this paper, on 5 to 8 mg. of tissue and by the accepted macro-methods is suggested by the agreement of our findings for the "normal" liver (5.7 ± 0.8 g. lipid per 100 g. wet liver for the mean $\pm$ standard deviation) and those of Ralli and her co-workers (4) who concluded that the concentration of total lipid in the normal human liver will probably not exceed 8 g. per 100 g. wet liver. Other workers have reported similar values for the normal human liver (Man and coworkers [13], Thannhauser and Reinstein [14], and Rourke and Stewart [2]).

Histological examination of needle specimens taken from various sites in the liver has shown that relative uniformity in the distribution of fat does exist even in cirrhotic livers. Chaikoff and Kaplan (15) reported that in depancreatized dogs the distribution of fat in the liver may be uneven and that fatty as well as normal portions may exist simultaneously in the same liver. Such a situation has not, however, been observed in our material.

Inspection of the results obtained by chemical analysis shows that the lipid content of the specimens obtained from any one liver (*1*) varies for each individual, (*2*) is dependent upon the histopathologic alteration, (*3*) shows greater variation in those livers with a high lipid content (Fig. 9) and that (*4*) this variation depends to some extent on the presence or absence of cirrhosis. Statistical analysis has shown, however, that the variances for the four groups are essentially homogeneous. It can therefore be concluded that it is as accurate, on a percentage basis, to estimate the lipid concentration of the entire liver from a single specimen, whether cirrhosis is present or absent, or the fat content high or low.

Using the data obtained in this study, it is possible to estimate from a needle specimen the lipid concentration of the entire liver with a determined

error. The best estimate is the concentration of lipid found in the specimen itself and its error may be conveniently expressed as the 5 per cent fiducial limits, which indicate the limits between which the lipid concentration of the liver will probably be 19 out of 20 times (16). In calculating these limits the variance of the group is used since this is the best estimate of the variance of the individual and the grouping is known from histological examination of the specimen. Thus, for a non-fatty, non-cirrhotic liver (*i.e.*, "normal") the lipid concentration in the whole liver is within the range of ± 25 per cent of the value found from the micro-analysis of a single sample. The limits for the remaining groups are summarized in Table VI. When two samples are analysed, a more precise estimate can be obtained.

If some therapy is given that is expected to modify the lipid content of the liver, it will be necessary to know whether any change observed is greater than that expected by chance. The "t" test (17) has been performed on the differences in the mean values, using the variance of the group as the best estimate of the variance for any sample shown by histological examination to belong to that group. In the third column of Table VI is given the minimum change in lipid (expressed as a fraction of the control sample) which is necessary to indicate a decrease, at the 5 per cent level of significance, in the second sample. For example, if the initial sample from a fatty non-cirrhotic liver had a lipid concentration of 20 g. per 100 g. liver, then a decrease of 6.2 g. per 100 g. liver or greater would be necessary to ensure that the change would not have been likely on the basis of sampling variation. If the analysis is carried out on two biopsy specimens, taken simultaneously, then a smaller difference between the first and second samples will be significant (Column 4, Table VI).

T. K. With (18) showed that in man, analysis of a small sample of liver tissue does provide values which can be regarded as representative of the average concentration of Vitamin A in the liver, particularly of the right lobe. Specimens of liver obtained by the needle biopsy technique can also be regarded as representative of the lipid content of the liver as a whole within the limits stated above. Recognition of these limits is necessary if a valid interpretation of the results obtained is to be made.

SUMMARY

1. Methods for the chemical analysis of the percentage of dry matter, total lipids, total fatty acids and their iodine number, and phospholipids in specimens of liver tissue obtained by the needle biopsy technique are described.

2. Using a Vim-Silverman needle, 18 specimens have been taken from different sites in each of 25 post-mortem livers (13 non-fatty and 12 fatty livers). The samples have been examined histologically and their total lipid concentration determined chemically. The variation in the different specimens is reported and the effect of cirrhosis on the findings is discussed.

3. Relative uniformity in the distribution of lipids throughout the human liver has been observed. A good correlation was found between histological and chemical findings.

4. Approximate limits of error in estimating the lipid concentration of the whole liver from analysis of a single biopsy specimen are given.

5. Factors are given which may be used to determine whether the value for the lipid concentration of a repeat biopsy specimen indicates a significant change in the lipid concentration in the whole liver.

ACKNOWLEDGMENTS

The authors wish to acknowledge the assistance of Dr. H. Frank in the preliminary work and of Dr. H. Ryder in the statistical analysis of the data. They wish to express their gratitude to Dr. K. Schmidt-Nielsen for valued advice on the microchemical techniques employed.

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ANNOUNCEMENT OF MEETINGS

The 45th annual meeting of the American Society for Clinical Investigation will be held in Atlantic City, N. J., on Monday, May 4, 1953, with headquarters at the Chalfonte-Haddon Hall. The scientific session will begin at 9 a.m. at the Steel Pier Theater.

The annual meeting of the Association of American Physicians will be held at the Chalfonte-Haddon Hall on Tuesday, May 5, and the morning of Wednesday, May 6, 1953.

A COMPARISON OF THE EFFECTS OF *l*-NOR-EPINEPHRINE, SYNTHETIC *l*-EPINEPHRINE, AND U.S.P. EPINEPHRINE UPON CEREBRAL BLOOD FLOW AND METABOLISM IN MAN [1]

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(Submitted for publication November 17, 1952; accepted December 10, 1952)

Nor-epinephrine, a pressor amine known to exist normally in the mammalian body, is identical with epinephrine except for the absence of a methyl group in its chemical structure. Since methylation occurs readily in the body, it has been suggested that nór-epinephrine functions chiefly as a precursor to epinephrine (1–5). Nevertheless, it possesses potent pharmacologic properties which differ in many respects from those of epinephrine (6–9). Epinephrine raises systolic blood pressure, increases cardiac output and accelerates the heart rate; it constricts blood vessels in certain areas of the circulatory bed, dilates them in others, with an over-all effect of dilatation and a decrease in total peripheral vascular resistance. Nor-epinephrine increases systolic and diastolic blood pressure, has little or no effect on cardiac output and decreases cardiac rate. It constricts blood vessels in all areas of the circulation in which its effect has been studied.

Little is known, however, of the effects of nor-epinephrine and epinephrine upon the cerebral circulation in man. A clear understanding of their effects on this important segment of the circulation should provide information bearing on a number of physiologic, pathologic, and therapeutic questions. Because of the similarity of the hemodynamic changes in essential hypertension to those induced experimentally by the administration of *l*-nor-epinephrine, it has been suggested that *l*-nor-epinephrine may play an important role in the pathogenesis of essential hypertension (6). A measure of support for this hypothesis would be gained if nor-epinephrine-induced hypertension could be shown to be associated with changes in the cerebral circulation similar to those accompanying essential hypertension. If, on the other hand, *l*-nor-epinephrine affects the cerebral circulation in some unique or distinctive way, this information might be of some value in the clinical recognition and detection of certain hypertensive states associated with abnormally high concentrations of *l*-nor-epinephrine in the adrenal gland, *i.e.*, pheochromocytoma (10, 11). Furthermore, nor-epinephrine has been employed therapeutically as a pressor agent in a variety of conditions characterized by acute hypotension including operative, hemorrhagic, and traumatic shock; to maintain blood pressure after thoracolumbar sympathectomy; and in the circulatory collapse that may accompany acute myocardial infarction (12, 13). A clear understanding of its effect on the cerebral circulation would be essential to a proper assessment of its over-all therapeutic value in these conditions. Obviously, if the increase in systemic arterial blood pressure is accompanied by a disproportionate increase in cerebral vascular resistance, a further reduction in blood flow to the brain would ensue. Such an effect would, no doubt, detract from its usefulness in the treatment of these hypotensive states.

This report deals with studies made to determine the effect of *l*-nor-epinephrine, and *l*-epinephrine on the cerebral circulation in normal, healthy males. Since U.S.P. epinephrine has been shown to contain significant amounts of *l*-nor-epinephrine (14, 15), it was necessary to determine the effect of both U.S.P. epinephrine and uncontaminated synthetic *l*-epinephrine.

METHOD

The cerebral blood flow (CBF) was determined by the nitrous oxide method of Kety and Schmidt (16), as modi-

[1] Reviewed in the Veterans Administration and published with the approval of the Chief Medical Director. The statements and conclusions published by the authors are the result of their own study and do not necessarily reflect the opinion or policy of the Veterans Administration.

TABLE I

The effect of l-*nor-epinephrine on cerebral circulatory functions*

Subject	Age	Dosage μg.	CBF cc./min./100 gms. Control	CBF cc./min./100 gms. Exper.	CVR mm. Hg/cc./min./100 gms. Control	CVR mm. Hg/cc./min./100 gms. Exper.	CMR O_2 cc./min./100 gms. Control	CMR O_2 cc./min./100 gms. Exper.	MABP mm. Hg Control	MABP mm. Hg Exper.
H. W.	29	1,000	54	49	1.35	2.02	4.28	4.35	73	99
A. R. H.	21	1,000	58	45	1.36	2.00	4.80	4.22	79	90
H. L. B.	27	1,400	61	52	1.44	2.09	4.10	5.16	88	109
W. C. C.	40	1,000	67	54	1.10	1.94	4.11	4.16	74	105
C. W.	26	800	42	33	1.90	3.06	3.02	2.86	80	101
A. M. M.	33	800	83	51	0.81	1.86	4.43	3.85	67	99
R. B.	23	800	50	34	1.72	3.09	4.02	2.96	86	105
W. L. P.	32	1,000	45	43	1.46	1.95	2.98	2.85	66	84
J. F. C.	27	1,000	60	43	1.15	2.58	3.68	3.07	69	111
R. H. H.	23	1,000	62	57	1.16	1.77	3.81	4.78	72	101
J. W. B.	21	700	83	56	0.96	1.64	4.93	3.84	80	92
G. D.	36	800	68	54	1.16	1.83	4.07	3.47	79	99
R. A. P.	24	800	63	59	1.11	1.47	3.91	3.85	70	86
Mean	28		61	48*	1.28	2.10*	4.01	3.80	76	99*
S.D.			11.94	8.05	.28	.48	.55	.71	6.73	8.07
S.E.			3.44	2.32	.08	.13	.15	.20	1.94	2.33
P value				<0.01		<0.01		>0.20		<0.01

* Indicates statistically significant change.

fied by Scheinberg and Stead (17). The cerebral oxygen consumption (CMR_{O_2}) was calculated from the CBF and the arterio-cerebral venous oxygen difference. The cerebral vascular resistance (CVR) was calculated from the CBF and the mean arterial blood pressure, which was measured directly in a peripheral artery, usually the femoral, with a damped mercury manometer. Arterial and cerebral venous blood samples were drawn simultaneously immediately before each blood flow determination. Blood oxygen and carbon dioxide content were determined manometrically (18). The cerebral blood flows were measured in the morning with the subject fasting and in the recumbent position. Immediately following a control determination, *l*-nor-epinephrine, synthetic *l*-epinephrine,[2] or U.S.P. epinephrine in oil was administered intramuscularly in amounts varying from 600 μg. to 1,400 μg. Fifteen or twenty minutes later, when

[2] Generous quantities of *l*-nor-epinephrine (Levophen) and *l*-epinephrine (Suprarenin) were supplied by Kenneth M. Smoot, Dept. of Medical Research, Winthrop-Stearns, Inc.

TABLE II

The effect of l-*nor-epinephrine on arterial and cerebral venous blood constituents*

Subject	Age	Dosage μg.	Arterial blood CO_2 content vol. % Control	Arterial blood CO_2 content vol. % Exper.	Arterial blood O_2 content vol. % Control	Arterial blood O_2 content vol. % Exper.	Internal jugular venous blood CO_2 content vol. % Control	Internal jugular venous blood CO_2 content vol. % Exper.	Internal jugular venous blood O_2 content vol. % Control	Internal jugular venous blood O_2 content vol. % Exper.	A-V O_2 Vol. % Control	A-V O_2 Vol. % Exper.	Oxygen extraction ratio ER O_2. % Control	Oxygen extraction ratio ER O_2. % Exper.
H. W.	29	1,000	42.23	40.13	20.07	20.94	49.63	48.81	12.15	12.07	7.92	8.87	39	42
A. R. H.	21	1.000	44.50	42.54	22.72	22.75	52.13	51.10	14.44	13.38	8.28	9.37	36	41
H. L. B.	27	1,400	45.48	39.13	20.38	22.37	51.31	48.73	13.58	12.43	6.80	9.94	33	44
W. C. C.	40	1,000	46.10	44.39	17.60	19.16	52.86	53.76	11.46	11.44	6.14	7.72	35	40
C. W.	26	800	44.91	43.39	21.25	21.82	51.68	51.37	14.05	13.14	7.20	8.68	34	40
A. M. M.	33	800	46.95	43.78	18.13	19.14	51.05	51.10	12.79	11.59	5.34	7.55	29	39
R. B.	23	800	49.26	48.09	20.38	20.74	56.96	56.99	12.34	12.01	8.04	8.73	39	42
W. L. P.	32	1,000	49.10	46.22	18.69	19.65	55.47	54.00	12.06	12.43	6.63	7.22	35	37
J. F. C.	27	1,000	47.94	45.45	17.97	19.17	53.38	51.69	11.84	12.02	6.13	7.15	34	37
R. H. H.	23	1,000	51.88	47.94	19.16	20.26	53.40	55.67	13.00	11.87	6.16	8.39	32	41
J. W. B.	21	700	51.41	48.50	18.94	19.54	55.54	55.08	14.15	12.69	4.79	6.85	25	35
G. D.	36	800	48.85	48.82	18.67	19.08	54.24	54.76	12.68	12.66	5.99	6.42	32	34
R. A. P.	24	800	47.86	46.95	18.36	18.36	53.07	53.45	12.15	11.82	6.21	6.54	34	36
Mean	28		47.42	45.03*	19.41	20.23*	53.13	52.81	12.82	12.27*	6.59	7.96*	34	39*
S.D.			2.66	3.02	1.41	1.33	1.96	2.46	.92	.55	1.00	1.07	3.62	2.92
S.E.			.76	.87	.40	.38	.56	.71	.26	.16	.28	.31	1.04	.84
P value				<0.01		<0.01		>0.30		<0.01		<0.01		<0.01

* Indicates statistically significant change.

TABLE III

The effect of synthetic l-epinephrine on cerebral circulatory functions

Subject	Age	Dosage μg.	CBF cc./min./100 gms.		CVR mm. Hg/cc./min./100 gms.		CMR O_2 cc./min./100 gms.		MABP mm. Hg	
			Control	Exper.	Control	Exper.	Control	Exper.	Control	Exper.
B. G. T.	25	1,000	43	45	1.76	1.66	4.20	3.61	76	75
J. W. F.	27	1,000	57	64	1.33	1.07	3.82	4.34	76	69
E. B.	29	600	60	59	1.27	1.24	3.77	4.08	76	73
M. L. A.	33	1,000	50	56	1.62	1.32	3.84	4.35	81	74
J. E. B.	33	800	68	67	1.04	0.95	5.20	4.95	71	66
J. L. P.	27	1,400	49	49	1.31	1.27	4.01		64	62
B. W. M.	28	1,000	68	54	1.04	1.22	3.82	2.42	71	66
T. R. E.	33	1,000	68	58	1.07	1.24	4.09	3.34	73	72
J. W. N.	28	1,000	68	76	1.10	0.88	4.39	5.02	75	68
E. R. B.	32	1,000	60	54	1.32	1.35	4.57	3.77	79	73
C. E. B.	30	1,400	69	69	1.23	1.13	3.80	4.28	85	78
E. L. M.	35	1,200	40	38	1.70	1.60	2.68	2.56	68	61
J. M. H.	22	1,000	98	97	0.72	0.69	4.51	4.37	71	67
I. W.	23	600	95	78	0.65	0.78	5.28	4.18	62	61
E. L.	24	1,200	95	79	0.87	1.01	4.40	5.79	83	80
Mean	29		66	63	1.20	1.16	4.16	4.08	74	70*
S.D.			17.57	14.73	.31	.26	.60	.87	6.30	5.76
S.E.			4.69	3.93	.08	.07	.16	.24	1.68	1.54
P value				>0.10		>0.30		>0.50		<0.01

* Indicates statistically significant change.

the blood pressure had become stabilized, the cerebral blood flow measurement was repeated. Previous experience with the intramuscular administration of *l*-nor-epinephrine had shown that a consistent pressor response occurs when the site of injection is massaged immediately after the administration of the drug. The blood pressure gradually rises for 15 to 20 minutes, becomes stabilized at an essentially constant level for an additional 10 to 20 minutes, and then gradually returns to normal. The second cerebral blood flow in each case was measured during the stable plateau period. The mean of four blood pressure measurements made immediately preceding and four made immediately following the blood flow determination was used as the mean arterial pressure during the

TABLE IV

The effect of synthetic l-epinephrine on arterial and cerebral venous blood constituents

Subject	Age	Dosage μg.	Arterial blood				Internal jugular venous blood				A-V O_2 Vol. %		Oxygen extraction ratio ER O_2, %	
			CO_2 content vol. %		O_2 content vol. %		CO_2 content vol. %		O_2 content vol. %					
			Control	Exper.	Control	Exper.	Control	Exper.	Control	Exper.	Control	Exper.	Control	Exper.
B. G. T.	25	1,000	44.20	45.35	21.80	21.72	53.23	53.25	12.03	13.69	9.77	8.03	45	37
J. W. F.	27	1,000	43.59	41.27	18.35	18.53	49.73	48.26	11.65	11.75	6.70	6.78	37	37
E. B.	29	600	47.23	46.77	18.15	18.42	52.73	53.76	11.87	11.51	6.28	6.91	35	38
M. L. A.	33	1,000	47.73	47.81	20.09	19.72	55.05	55.70	12.40	11.95	7.69	7.77	38	39
J. E. B.	33	800	45.31	46.19	21.15	21.46	52.66	53.59	13.50	13.77	7.65	7.69	36	36
J. L. P.	27	1,400	47.18		21.27		55.49		13.07		8.20		38	
B. W. M.	28	1,000	46.20	45.52	20.31	20.57	51.10	50.87	14.68	16.08	5.63	4.49	28	22
T. R. E.	33	1,000	43.34	46.01	17.95	17.95	49.48	51.12	11.93	12.19	6.02	5.76	34	32
J. W. N.	28	1,000	49.30	50.10	18.01	17.88	55.64	56.44	11.54	11.28	6.47	6.60	36	37
E. R. B.	32	1,000	46.33	46.27	20.98	21.01	53.43	53.18	13.36	14.03	7.62	6.98	36	33
C. E. B.	30	1,400	48.46	47.50	17.26	17.52	53.89	53.80	11.75	11.31	5.51	6.21	32	35
E. L. M.	35	1,200	44.37	43.16	15.50	16.25	50.79	49.50	8.80	9.50	6.70	6.75	43	42
J. M. H.	22	1,000	47.21	48.35	17.73	17.69	52.11	53.44	12.92	13.19	4.81	4.50	27	25
I. W.	23	600	48.25	49.58	17.72	17.89	53.12	55.18	12.16	12.52	5.56	5.37	31	30
E. L.	24	1,200	42.54	42.28	17.85	18.22	47.59	50.17	13.21	10.89	4.64	7.33	26	40
Mean	29		46.08	46.15	18.94	18.92	52.40	52.73	12.32	12.40	6.62	6.51	35	35
S.D.			2.01	2.47	1.77	1.61	2.23	2.32	1.26	1.58	1.33	1.08	5.22	5.47
S.E.			.53	.68	.47	.44	.59	.64	.33	.43	.35	.30	1.39	1.51
P value				>0.50		>0.05		>0.10		>0.50		>0.50		>0.50

* Indicates statistically significant change.

TABLE V

The effect of U.S.P. epinephrine on cerebral circulatory functions

Subject	Age	Dosage μg.	CBF cc./min./100 gms.		CVR mm. Hg/cc./min./100 gms.		CMR O_2 cc./min./100 gms.		MABP mm. Hg	
			Control	Exper.	Control	Exper.	Control	Exper.	Control	Exper.
C. E. L.	36	800	79	59	0.89	1.20	4.78	4.52	70	71
J. B. H.	39	1,000	42	50	1.83	1.34	2.73	3.38	77	67
J. C. H.	30	800	58	61	1.28	1.05	2.91	3.58	74	64
B. E. C.	38	800	69	51	1.04	1.43	5.44	4.12	72	73
W. M. J.	34	1,200	97	84	0.76	0.82	5.07	4.10	74	69
J. J.	32	1,200	67	69	1.00	0.90	3.93	3.53	67	62
R. A. A.	30	1,000	80	84	1.01	0.88	3.76	5.39	81	74
E. F. J.	31	1,000	85	94	0.99	0.84	5.28	5.59	84	79
E. L. C.	28	1,000	71	63	0.95	1.00	4.54	3.57	68	64
N. G. W.	41	1,000	76	73	1.09	0.99	4.59	4.41	83	72
J. M. M.	25	1,000	69	64	1.28	1.28	4.02	4.18	89	92
A. A. B.	34	1,000	50	49	1.74	1.69	3.62	3.26	87	83
C. D. D.	25	1,000	82	73	0.96	1.05		4.67	79	77
Mean	33		71	67	1.14	1.11	4.22	4.18	77	73*
S.D.			14.24	13.52	.30	.25	.84	.70	6.91	8.10
S.E.			4.11	3.90	.08	.07	.25	.20	1.99	2.33
P value				>0.10		>0.50		>0.50		<0.01

* Indicates statistically significant change.

procedure. Blood pressure values before and after measurement of CBF did not vary more than a few millimeters in any case.

RESULTS

l-Nor-epinephrine. The data (Tables I and II) indicate that *l*-nor-epinephrine is a potent cerebral vasoconstrictor. The disproportionate increase (64 per cent) in calculated mean CVR ($p = < 0.01$) above MABP (31 per cent), eventuates in a 21 per cent reduction in CBF ($p = < .01$). Nevertheless, the CMR_{O_2} is unaffected because the fall in CBF is compensated by an increase in the oxygen extraction ratio (34 to 39 per cent) and in mean arterio-cerebral venous oxygen difference (6.59 vol. per cent to 7.96 vol. per cent) ($p = < .01$). The increase in A–V oxygen difference is the re-

TABLE VI

The effect of U.S.P. epinephrine on arterial and cerebral venous blood constituents

Subject	Age	Dosage μg.	Arterial blood: CO_2 content vol. %		Arterial blood: O_2 content vol. %		Internal jugular venous blood: CO_2 content vol. %		Internal jugular venous blood: O_2 content vol. %		A-V O_2 Vol. %		Oxygen extraction ratio ER O_2, %	
			Control	Exper.	Control	Exper.	Control	Exper.	Control	Exper.	Control	Exper.	Control	Exper.
C. E. L.	36	800	47.37	46.24	17.93	18.63	53.77	52.73	11.88	11.91	6.05	6.72	34	36
J. B. H.	39	1,000	47.75	49.48	18.80	18.46	54.77	56.06	12.30	11.71	6.50	6.75	35	37
J. C. H.	30	800	46.74	47.50	17.03	16.71	51.88	53.72	12.01	10.84	5.02	5.87	29	35
B. E. C.	38	800	47.83	48.73	20.36	20.38	55.93	56.45	12.47	12.30	7.89	8.08	39	40
W. M. J.	34	1,200	48.17	49.68	17.85	17.70	53.62	55.34	12.62	12.82	5.23	4.88	29	28
J. J.	32	1,200	39.38	40.27	15.65	15.67	45.74	45.53	9.78	10.56	5.87	5.11	38	33
R. A. A.	30	1,000	52.10	50.54	18.38	20.02	56.75	56.55	13.67	13.60	4.71	6.42	26	32
E. F. J.	31	1,000	47.39	48.55	20.00	19.95	54.02	54.40	13.79	14.00	6.21	5.95	31	30
E. L. C.	28	1,000	44.16	46.91	16.34	15.37	51.20	52.60	9.94	9.69	6.40	5.68	39	37
N. G. W.	41	1,000	48.32	49.09	16.75	16.32	54.80	56.00	10.71	10.28	6.04	6.04	36	37
J. M. M.	25	1,000	48.46	49.00	16.68	16.63	54.49	55.93	10.84	10.10	5.84	6.53	35	39
A. A. B.	34	1,000	46.75	46.90	20.23	20.13	53.77	54.46	13.03	13.48	7.23	6.65	36	33
C. D. D.	25	1,000		48.03		21.10		53.82		14.70		6.40		30
Mean	33		47.04	47.76	18.00	18.24	53.40	54.12*	11.92	12.00	6.08	6.24	34	34
S.D.			2.87	2.46	1.52	1.88	2.72	2.79	1.28	1.57	.85	.77	4.07	3.58
S.E.			.86	.71	.45	.54	.82	.80	.38	.45	.25	.22	1.22	1.03
P value				>0.05		>0.50		<0.02		>0.30		>0.50		>0.40

* Indicates statistically significant change.

sult of a significant increase in arterial O_2 content (19.41 vol. per cent to 20.23 vol. per cent) and a decrease in cerebral venous oxygen content (12.82 vol. per cent to 12.27 vol. per cent). Mean arterial CO_2 content decreased significantly (47.42 vol. per cent to 45.03 vol. per cent) apparently as a result of mild hyperventilation.

Synthetic l-epinephrine. In contrast to *l*-nor-epinephrine, this substance had no significant effect on mean cerebral hemodynamics or metabolism (Table III); nor were any changes in blood gases noted (Table IV). The only change of significance was a small but consistent reduction in MABP from 74 mm. Hg to 70 mm. Hg ($p = < 0.01$).

U.S.P. epinephrine. The results of studies made with U.S.P. epinephrine are listed in Table V and Table VI. With one exception they are identical with the findings in the synthetic epinephrine series, *i.e.*, a slight but significant reduction in MABP (77 mm. Hg to 73 mm. Hg; $P = < .01$) without changes in CBF, CVR, CMR_{O_2}, arterial or venous oxygen content. With both synthetic and U.S.P. epinephrine there was an increase in mean cerebral venous CO_2 content which on statistical analysis proved to be of significance only with U.S.P. epinephrine.

DISCUSSION

These experiments demonstrate that the administration of *l*-nor-epinephrine results in powerful cerebral vasoconstriction in normal healthy males. The increase in cerebral vascular resistance induced by this substance exceeds the accompanying increase in perfusion pressure; as a consequence, the blood flow to the brain is diminished. This decrease in CBF, however, does not eventuate in a reduction in cerebral oxygen consumption because of the compensatory increase in the extraction of oxygen from arterial blood by the brain, *i.e.*, the arterio-cerebral venous oxygen difference increases.

The increase in cerebral vascular resistance is most likely due to a direct vasoconstricting effect of *l*-nor-epinephrine upon cerebral vessels. However, this vasoconstricting action could be augmented by changes in O_2 tension, CO_2 tension or pH of the arterial blood (19). King, Sokoloff, and Wechsler (20) have shown that the intravenous infusion of *l*-nor-epinephrine is not accompanied by a change in pH of the blood. However, a rise in arterial oxygen content and a fall in arterial carbon dioxide content occurs when the drug is administered by either the intramuscular or intravenous route. These changes in blood gases are attributed to the mild hyperventilation that was observed in most of the subjects. However, they are not of sufficient magnitude to account for the marked increase in cerebral vascular resistance and could contribute only in a small way, if at all, to the total increase in cerebral vascular resistance (19). It seems very unlikely that reduced arterial CO_2 tension is causally related to the cerebral vasoconstriction induced by *l*-nor-epinephrine although it is possible that slight changes in blood CO_2 tension incident to hyperventilation might tend to augment the direct vasoconstricting effect of the drug. There is the further possibility that acute hypertension resulting from the administration of *l*-nor-epinephrine may be associated with an increase in intracranial pressure, and in this way further augment the resistance to blood flow in the brain.

These studies disclosed a striking difference between the cerebral circulation in essential hypertension and the acute hypertension induced by *l*-nor-epinephrine. In the former there are parallel increases in cerebral vascular resistance and blood pressure; the CBF, therefore, remains unaltered (21). In the latter, the augmentation of CVR is out of proportion to the increase in blood pressure; the blood flow is reduced. This does not, however, constitute strong evidence against the suggestion that *l*-nor-epinephrine may be involved in the pathogenesis of essential hypertension, since the hypertension noted in this study was acute and the state of the cerebral circulation under these circumstances not necessarily comparable to that in chronic essential hypertension. Furthermore, there is the possibility, indicated above, that indirect and secondary factors not present in essential hypertension may augment the increase in cerebral vascular resistance consequent to the acute administration of *l*-nor-epinephrine.

The demonstration that the pressor response of *l*-nor-epinephrine is accompanied by a reduction in cerebral blood flow in normotensive subjects suggests that this drug should be used with cau-

tion as a therapeutic agent in acute hypotensive states when a reduction in blood flow to the brain may not be desirable.

Other vital segments of the circulation have been shown to be similarly affected by the administration of this substance. Renal and hepatic blood flow are diminished and calculated renal and splanchnic vascular resistance increased during the administration of nor-epinephrine (22–25). Until it can be shown that the cerebral, as well as the renal and hepatic, circulations in patients with acute hypotension respond differently to *l*-nor-epinephrine than in normotensive subjects, its use here should be considered potentially harmful.

These studies with epinephrine confirm the previous assumption that the quantities of nor-epinephrine present in U.S.P. epinephrine do not alter the effect of the latter (5), and they demonstrate yet another difference in the hemodynamic effects of nor-epinephrine and epinephrine. The intramuscular administration of epinephrine leads to a slight reduction in mean arterial blood pressure but does not alter cerebral vascular resistance, cerebral blood flow or cerebral oxygen consumption. These findings are in disagreement with those of King, Sokoloff, and Wechsler (20) who found that the intravenous infusion of epinephrine was accompanied by an increase in blood pressure, cerebral blood flow and cerebral oxygen consumption; cerebral vascular resistance was not changed. The differences in blood flow and blood pressure responses can be adequately accounted for by the difference in mode of administration of epinephrine in the two studies. Our experimental blood flows were determined 25 to 30 minutes after the intramuscular administration of epinephrine when there were clear-cut clinical manifestations of its effect such as tremulousness, tachycardia, palpitation, pallor, excitement, and apprehension; no special attempt was made to produce a pressor response in the subjects. In the studies of King, Sokoloff, and Wechsler (20) epinephrine was administered by intravenous infusion, the rate of which was adjusted to provide a pressor response before the experimental studies were done. The concentration of circulating epinephrine obtained as a result of the slow absorption from an intramuscular deposit of epinephrine in oil was no doubt inadequate to cause an increase in cardiac output of sufficient magnitude to overcome the over-all vasodilating effect of epinephrine. Since epinephrine by either route of administration does not alter cerebral vascular resistance, its effect upon the blood flow to the brain will depend directly upon its effect upon blood pressure.

The results of the two studies regarding cerebral oxygen utilization, however, cannot be attributed to the difference in mode of administration. Excitement, apprehension, palpitation, tremulousness, pallor, etc., occurred in most of the subjects after the intramuscular administration of *l*-epinephrine and U.S.P. epinephrine, and in many instances these manifestations were quite prominent. Cerebral oxygen consumption, however, was unaffected by these agents. No change in mean CMR_{O_2} was observed in groups who received either *l*-epinephrine or U.S.P. epinephrine. Nor were there individual instances in either group in which there appeared to be a relationship between changes in cerebral oxygen consumption and the severity of the subjective sensations experienced by the subjects.

The results in this respect are similar to those of Scheinberg and Stead (17) who found that cerebral blood flow and cerebral oxygen consumption were unaltered by apprehension and anxiety in normal young males, and they tend to support the suggestion of Scheinberg (26) that cerebral metabolism normally functions at nearly its maximum rate. Apprehension and anxiety, whether occurring spontaneously or induced by the intramuscular administration of epinephrine, do not appear to be associated with changes in cerebral oxygen consumption.

SUMMARY

1. The effect of the intramuscular administration of *l*-nor-epinephrine, synthetic *l*-epinephrine, and U.S.P. epinephrine on cerebral circulatory and metabolic function has been determined in normal healthy males.

2. *l*-Nor-epinephrine increases mean arterial blood pressure and reduces cerebral blood flow in normotensive males by virtue of its potent constricting effect upon cerebral blood vessels. Cerebral oxygen utilization is not altered.

3. Epinephrine, when administered intramuscularly in dosage of from 600 μg. to 1,400 μg.,

slightly lowers mean arterial blood pressure; does not affect cerebral blood flow, cerebral vascular resistance or cerebral oxygen utilization.

4. The cerebrovascular effects of synthetic *l*-epinephrine and U.S.P. epinephrine are identical.

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THE EFFECT OF AN ARTERIOVENOUS FISTULA ON RENAL HEMODYNAMICS AND ELECTROLYTE EXCRETION [1]

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Patients with a compressible fistula between peripheral artery and vein provide a unique opportunity to study the reactions of the body to certain abrupt changes in the circulation. As has been said of the patient with congestive heart failure (1), the subject with an arteriovenous fistula is literally bleeding into his large veins. Opening or closing a fistula causes an immediate and marked change in peripheral resistance and a shift in the distribution of blood inside the vascular tree. This in turn elicits numerous compensatory adjustments (2–4).

It was of interest to examine the behavior of the kidneys in this situation for two reasons. First, the renal circulatory pattern in patients with arteriovenous fistulas has not been described. Secondly, it has been suggested that the kidneys retain or excrete sodium in response to alterations in the "effective" distribution of blood (5, 6). The present study permitted this hypothesis to be tested. The data presented show that glomerular filtration rate, renal blood flow and renal venous pressure do not change when a fistula is closed or opened, but demonstrate that the renal excretion of sodium is augmented when an arteriovenous shunt is compressed.

METHODS

Subjects were 17 young male casualties of the Korean war, treated on the Vascular Surgery Service of the Walter Reed Army Hospital. Each patient had a large arteriovenous fistula in the neck, shoulder, arm or leg, created by a penetrating wound received one and a half to five months previously. The diameters of the shunts as estimated at operation varied from 3 millimeters to 20 millimeters. No patient exhibited the signs or symptoms of congestive heart failure.

Patients were studied in the morning, in the postabsorptive state, while lying supine. After awaking, they were not permitted to get up and walk about before being wheeled in the supine position to the laboratory at 8 A.M. In all except four instances the subjects drank 240 cc. of water or hypotonic (0.14 per cent) saline every hour or half hour from 6:00 A.M. until the conclusion of the procedure, in order to insure a copious flow of urine. In seven experiments, including all those with initial urinary flows of less than 4.0 cc. per minute and collection periods of less than 20 minutes, urine was collected through an indwelling multi-eyed urethral catheter and the bladder was rinsed with distilled water and air. In the other studies, in which relatively high urinary flows and long collection periods minimized errors in the collection of urine, patients were permitted to stand briefly and void voluntarily. Clearances of inulin (7) and para-aminohippurate (8) were determined during constant infusion following a priming dose. Renal blood flow was calculated from the clearance of para-aminohippurate and venous hematocrit. In nine patients the clearance of endogenous creatinine was determined using a modification of the analytical technique of Bonsnes and Taussky (9). Concentrations of sodium and potassium in serum and urine were determined with a flame photometer. The method of Schales and Schales (10) was used to determine chloride in the urine of two patients. At intervals of two to five minutes throughout the procedure arterial pressure was measured with a sphygmomanometer and the pulse was counted for 30 seconds at the wrist. The mean blood pressure was arbitrarily taken to equal diastolic pressure plus ⅓ of the pulse pressure (11). All data were analyzed for significance by the Fisher "t" test.

Following a *"CONTROL"* period of at least 30 minutes, consisting of two or more clearance periods, the fistula was occluded by direct manual pressure over the fistula itself or the artery proximal to it, in such a fashion as to eliminate the characteristic bruit and to produce a definite elevation in diastolic blood pressure and slowing of the cardiac rate. *"COMPRESSION"* was maintained for 20 to 50 minutes while one to four collections of urine were made. After this the fistula was released and measurements were continued for another 20 to 50 minutes of *"RECOVERY."* After operative repair of the fistula, the same procedure was repeated in 12 patients, substituting pressure over the operative site or the opposite artery for compression of the previously existing fistula.

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RESULTS

1. *Blood pressure and pulse rate* (Table I)

Acute occlusion of an arteriovenous fistula in every instance caused an immediate and sustained increase in diastolic blood pressure, averaging 15 mm. Hg. Systolic pressure rose less or not at all, and in four patients, actually fell slightly. During compression, therefore, the calculated mean arterial pressure increased, and the pulse pressure diminished in every case. The average rise in mean arterial pressure was 11 mm. Hg, with values ranging from 4 to 20 mm. Hg. In general, those patients with the largest arteriovenous communications, as estimated by the surgeon at the time of operation, demonstrated the most marked elevation of mean blood pressure when their fistulas were compressed. The cardiac rate of all patients slowed when the fistula was compressed and became more rapid when the shunt was released (Nicoladoni-Branham sign) (12, 13).

2. *Renal hemodynamics* (Tables I and II, Figure 1)

In all subjects except two (H. B. and F. R.) the clearance of inulin or endogenous creatinine was within normal limits. Renal blood flow was normal in all but one patient (R. J.), who had a bifid ureter demonstrated by intravenous pyelography, but no other evidence of renal disease. These values were not changed significantly by surgical repair of the fistula. Acute occlusion and release of an arteriovenous shunt produced no

TABLE I

Summary of data obtained in the seventeen patients studied pre-operatively

Patient	Location of fistula	Procedure	Duration *minutes*	Number of periods	B. P. *mm. Hg*	Mean press. *mm. Hg*	Pulse rate *beats/min.*	G.F.R.* *cc./min. per 1.73 m.²*	R.B.F. *cc./min. per 1.73 m.²*	Urine flow *cc./min.*	$U_K V$† *mEq./min.*	$U_{Na} V$†† *mEq./min.*	E/F_{Na}§
		Control	44	3	91/53	66	88	146	1040	1.5		0.151	0.007
1. G. B.	Femoral	Compression	36	3	99/67	78	72	142	1100	2.3		0.295	0.014
		Recovery	48	3	97/57	70	85	132	1033	1.6		0.171	0.008
		Contr.	40	3	94/62	73	77	129	1080	5.2	0.051	0.231	0.011
2. G. M.	Femoral	Compr.	50	5	95/70	78	71	127	1110	3.1	0.045	0.394	0.020
		Recov.	26	3	97/69	78	78	126	1058	3.3	0.042	0.310	0.016
		Contr.	59	2	120/69	86	73	126	1098	5.0		0.320	0.015
3. R. C.	Femoral	Compr.	31	1	121/82	95	58	132	1065	4.4		0.512	0.022
		Recov.	31	1	121/64	83	76	148	1185	2.4		0.331	0.013
		Compr.	30	1	121/84	96	54	138	1082	3.7		0.392	0.016
		Contr.	113	2	119/73	88	77	*117*		17.9	0.035	0.199	0.012
		Compr.	32	1	117/80	92	59	*111*		19.5	0.021	0.318	0.021
		Recov.	60	2	117/69	85	77	*100*		11.6	0.022	0.209	0.021
		Contr.	31	2	112/41	65	109	112	1023	3.0		0.218	0.014
4. W. G.	Femoral	Compr.	21	2	105/65	78	94	117	1082	4.6		0.287	0.018
		Recov.	33	3	115/45	68	109	109	1098	3.3		0.180	0.012
		Contr.	41	3	104/41	62	75	117	648	6.7		0.160	0.009
5. R. J.	Sub-clavian	Compr.	33	2	108/76	87	52	104	554	5.2		0.200	0.013
		Recov.	44	4	111/48	69	76	113	660	5.2		0.158	0.009
		Contr.	38	3	134/53	80	69	143	830	2.1	0.029	0.298	0.012
6. J. C.	Femoral	Compr.	44	3	130/80	97	56	129	831	2.6	0.024	0.321	0.014
		Recov.	35	3	136/51	79	70	134	755	2.0	0.022	0.260	0.011
		Compr.	10	1	143/88	106	58	125	816	3.5		0.343	0.016
		Contr.	51	2	102/61	75	69	103	742	3.9		0.115	0.007
7. G. S.	Carotid	Compr.	39	1	108/78	88	49	110	875	6.8		0.202	0.011
		Recov.	41	1	105/59	74	71	97	723	3.4		0.091	0.008
		Contr.	72	3	131/80	97	76	111	785	13.0		0.370	0.023
8. J. F.	Brachial	Compr.	31	1	127/88	101	64	97	653	13.6		0.313	0.023
		Recov.	51	2	123/78	93	77	121	760	14.9		0.324	0.019
		Compr.	36	3	118/84	95	77	108	704	1.2		0.196	0.012
		Recov.	33	3	117/78	91	85	111	686	1.1		0.180	0.011
		Compr.	36	3	125/92	103	74	102	655	1.1		0.223	0.014

TABLE I—*Continued*

Patient	Location of fistula	Procedure	Duration *minutes*	Number of periods	B. P. *mm. Hg*	Mean press. *mm. Hg*	Pulse rate *beats/min.*	G.F.R.* *cc./min. per 1.73 m.²*	R.B.F. *cc./min. per 1.73 m.²*	Urine flow *cc./min.*	U_KV† *mEq./min.*	$U_{Na}V$†† *mEq./min.*	E/F_{Na}§
		Contr.	32	3	104/64	77	71	137	1327	3.2		0.071	0.003
9. W. T.	Carotid	Compr.	46	4	108/73	85	66	131	1158	4.2		0.114	0.006
		Recov.	31	3	103/67	79	99	116	1245	1.3		0.122	0.007
		Contr.	87	2	105/60	75	70	*118*		5.6	0.144	0.280	0.015
10. D. B.	Popliteal	Compr.	45	1	111/68	82	62	*128*		2.6	0.190	0.514	0.026
		Recov.	45	1	115/63	80	72	*116*		5.2	0.178	0.368	0.020
		Contr.	29	1	98/52	67	62	*120*		8.5	0.135	0.132	0.006
11. J. T.	Thoraco-acromial	Compr.	30	1	99/66	77	56	*116*		5.3	0.113	0.184	0.009
		Recov.	60	2	97/59	72	61	*117*		4.1	0.108	0.152	0.007
		Contr.	135	4	116/45	69	78	*105*		7.8	0.075	0.127	0.008
12. E. B.	Femoral	Compr.	28	1	121/73	89	65	*94*		8.5	0.088	0.273	0.020
		Recov.	58	2	124/52	76	79	*111*		4.8	0.078	0.235	0.015
		Contr.	69	3	111/56	72	66	*100*		8.7	0.095	0.110	0.008
13. A. D.	Femoral	Compr.	20	1	114/73	87	50	*100*		9.6	0.103	0.147	0.011
		Recov.	30	1	114/58	73	68	*91*		6.1	0.087	0.123	0.010
		Contr.	57	2	116/45	69	76	*78*		9.8		0.252	0.023
14. H. B.	Popliteal	Compr.	29	1	113/62	79	56	*72*		13.5		0.345	0.034
		Recov.	20	1	118/48	71	78	*77*		8.4		0.250	0.023
		Contr.	50	2	126/76	93	63	*140*		12.6		0.182	0.009
15. H. F.	Popliteal	Compr.	20	1	132/82	99	54	*137*		11.1		0.228	0.012
		Recov.	28	1	125/75	92	65	*143*		6.0		0.228	0.011
		Contr.	65	2	100/52	68	85	*146*		7.9		0.079	0.004
16. W. R.	Femoral	Compr.	31	1	100/58	72	68	*144*		7.0		0.099	0.005
		Recov.	32	1	98/52	67	80	*151*		6.7		0.079	0.004
		Contr.	120	4	112/57	75	66	*82*		5.4	0.038	0.046	0.004
17. F. R.	Femoral	Compr.	26	1	111/78	89	58	*89*		7.1	0.036	0.085	0.007
		Recov.	26	1	116/61	79	71	*83*		5.6	0.029	0.042	0.004
		Contr.			111/59	76	76	118	926	6.8	0.075	0.185	0.011
Mean		Compr.			112/74**	87**	62**	115	913	7.0	0.077	0.267**	0.016**
		Recov.			113/61**	78**	78**	115	920	5.1	0.071	0.200**	0.012**

* Italicized figures indicate clearances of endogenous creatinine. All other values in this column are clearances of inulin.

** Indicates "highly significant" change from the preceding value ($P < 0.01$).

† U_KV = Urine concentration of potassium in mEq./cc. × Urine volume flow in cc./min. = milliequivalents of potassium excreted in the urine per minute time.

†† $U_{Na}V$ = Urine concentration of sodium in mEq./cc. × Urine volume flow in cc./min. = milliequivalents of sodium excreted in the urine per minute time.

§ $E = U_{Na}V$. F_{Na} = Plasma concentration of sodium in mEq./cc. × Glomerular filtration rate in cc./min. = mEq./min. of sodium filtered by the glomeruli. Hence E/F_{Na} represents the ratio of excreted to filtered sodium.

consistent or significant change in the clearances of inulin, endogenous creatinine chromogen, or PAH; the filtration fraction, therefore, was also unaffected. The venous hematocrit was similarly unaltered. The left renal vein was catheterized in six patients (patients 12 to 17, Table I), in order to measure its pressure directly. Renal venous pressure was within normal limits (6 to 10 mm. Hg) (14) and was unaltered by temporary closure or opening of the arteriovenous fistula.

Since renal blood flow remained essentially unchanged when a fistula was occluded, despite a rise in the mean arterial pressure perfusing the kidneys, the total renal vascular resistance must have increased. If the data of Table I are analyzed according to the concepts of Gomez (15) it is apparent that this was accomplished largely by an increase in renal "afferent" arteriolar resistance.

3. *Excretion of electrolytes and water*

In contrast to the relative constancy of renal blood flow and glomerular filtration rate, the excretion of sodium increased significantly ($P < 0.01$) when the fistula was compressed in every

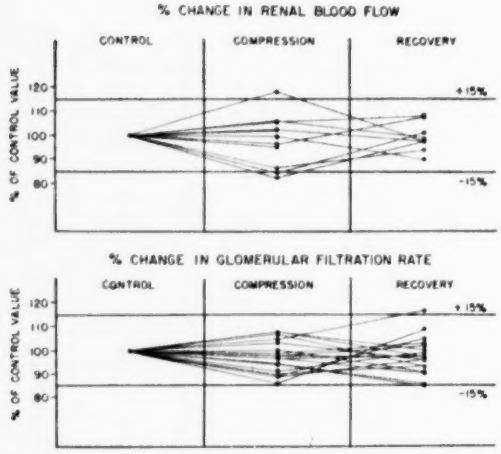

FIG. 1

TABLE II

Comparison of pre- and post-operative glomerular filtration rate and renal blood flow

Pt.	GFR* cc./min./1.73 m.² pre	GFR* cc./min./1.73 m.² post	RBF cc./min./1.73 m.² pre	RBF cc./min./1.73 m.² post
G. B.	146	137	1,040	1,194
P. M.	129	113	1,080	1,008
R. C.	126	133	1,098	1,455
W. G.	112	102	1,023	874
R. J.	117	112	648	635
J. C.	143	129	830	914
G. S.	139	112	923	1,030
J. F.	111	126	785	810
Mean	128	120	928	990

* Clearances of inulin. Each value represents the average of three collection periods.

There is no consistent or significant difference between the values before and after repair of an A-V fistula.

experiment except one (Table I, Figure 2). The increase ranged from 0.020 milliequivalents per minute to 0.234 milliequivalents per minute and the average rate of sodium excretion during the period of compression was 144 per cent of control. The effect could not be quantitatively correlated with the size of the shunt as estimated at operation or with the magnitude of the response of the blood pressure to occlusion. An augmented excretion of sodium was apparent in urine collected 10 to 15 minutes after occlusion of the fistula and continued throughout the compression period. After the fistula was released, sodium excretion fell toward control values in all but two instances. In two patients the urinary excretion of chloride was measured and found to parallel that of sodium. Following surgical repair of the

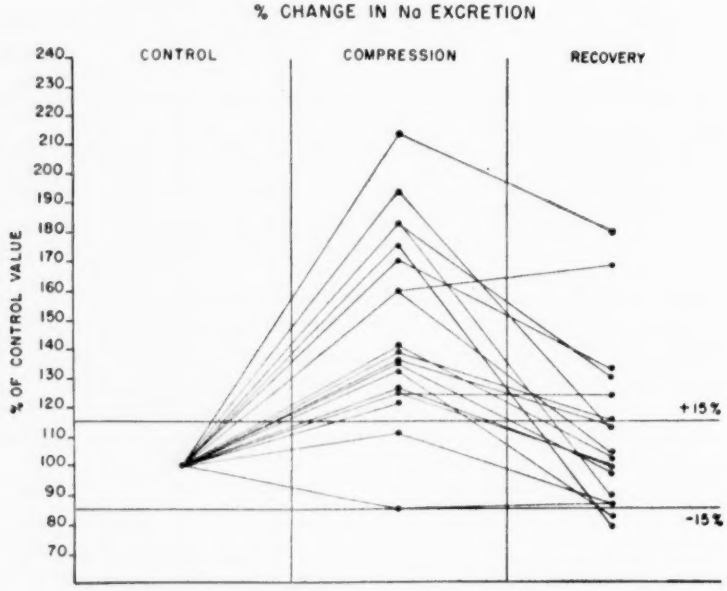

FIG. 2

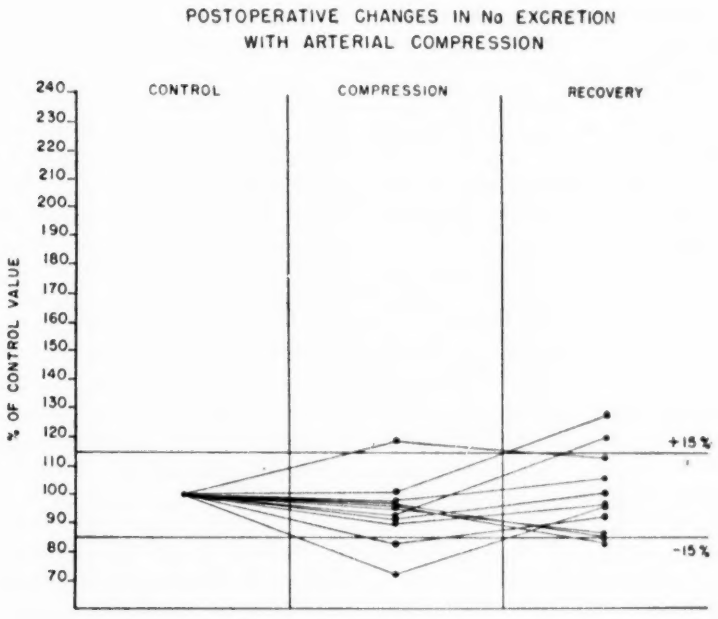

FIG. 3

fistula (Figure 3), renal excretion of sodium was unchanged by manual occlusion of an artery at the operative site or in the opposite limb, a procedure which did not produce significant changes in cardiac output or arterial pressure (16).

The renal excretion of potassium, studied in six patients, was not consistently altered by temporary closure or release of an arteriovenous shunt.

The flow of urine increased in 12 of 19 experiments when the fistula was closed, and diminished during the recovery period in 16. Interpretation of these findings is complicated by the fact that most of the patients had been drinking and were in positive water balance, and by the well-known tendency for urinary flow to fall off toward the end of a long and sometimes tiring procedure (17).

DISCUSSION

It is pertinent to review briefly the effects upon the general circulation of closing and opening an arteriovenous fistula and to consider how they might influence the kidneys. When an arteriovenous shunt is occluded, the arterial tree is emptied more slowly and less completely, and mean arterial pressure rises. Pressures in the great veins, the right atrium, and the pulmonary vessels tend to fall as these regions become less distended with blood (3, 18–21). Cardiac output is decreased as the heart rate slows and stroke volume diminishes. When the fistula is opened, blood pours from arteries into veins. Despite an increase in cardiac output (3, 4, 19, 22–24), the arteries become less distended, and mean systemic arterial pressure falls, while the great veins become more swollen and pressures in the right atrium and pulmonary artery may increase (3, 18–20).

The effects of sudden changes in renal vascular pressures, similar to those occurring in the present experiments, on renal hemodynamics, urinary flow and electrolyte excretion have been extensively studied in isolated kidneys and anesthetized animals, and have recently been discussed in detail (25–27). The kidneys of such preparations, whether or not the renal nerves are intact, exhibit a remarkable ability to maintain a constant rate of blood flow and glomerular filtration, in the face of wide variations in mean arterial perfusing pressure. Renal blood flow and filtration rate were similarly unaffected in the present study by the changes in mean arterial pressure accompanying closure and release of an arteriovenous

fistula, so that in contrast to the reported response of other vascular beds (3, 28, 29), the renal vascular resistance regularly increased when a peripheral fistula was occluded and decreased when it was opened. Presumably this is a manifestation of the "autonomy" of the renal vasculature expressed, in intact subjects (30–32) as in the isolated kidney, primarily through changes in afferent renal arteriolar resistance.

When an arteriovenous fistula is occluded, arterial pulse pressure is diminished at the same time that the excretion of sodium is augmented. A narrowed pulse pressure was thought by Hooker (33) and Gesell (34) to result in antidiuresis and retention of chloride. More recently it has been shown by Selkurt (35) and by Goodyer and Glenn (36) that alterations in renal arterial pulse pressure *per se* are not necessarily followed by changes in the excretion of sodium. On the other hand, an increase in the mean arterial pressure perfusing one kidney has been reported by Selkurt to induce a unilateral diuresis of salt and water without greatly changing the glomerular filtration rate (35). In all subjects of the present study, the increase in sodium excretion produced by closing a fistula was accompanied by an elevation of the calculated mean arterial pressure. However, the increase in pressure was always much smaller than that required in Selkurt's experiments to bring about the change in sodium excretion. Furthermore, the changes in sodium excretion from patient to patient could not be correlated with the magnitude of the response of the blood pressure.

The fraction of filtered sodium rejected by the tubules and appearing in the urine (E/F_{Na}) increased in all patients except one when flow through the fistula was shut off. In the absence of a consistent increase in the clearance of inulin or endogenous creatinine, the increase in renal excretion of sodium might be construed to be the result of altered tubular behavior toward this ion, presumably because of changes in nervous, hormonal or intrinsic renal influences. Unfortunately present techniques for the measurement of glomerular filtration are not accurate enough to rule out completely the possibility that an undetected increase in the filtration of sodium might have occurred to account for the observed increment in sodium excretion.

The present findings might be considered an example of the regulatory role of the kidneys in opposing distortions in the volume and distribution of the fluids of the body. It has long been realized that renal excretion of salt and water must be conditioned by the volume of body fluids, as well as by their tonicity (37), and it has been reasoned that renal excretion or retention of sodium may be related to some function of the volume of the circulating blood (38). Others have suggested that diuresis may be stimulated by an increase, and depressed by a reduction, in the cardiac output (39). Studies of the effects of posture on diuresis support the view that the effective distribution of blood rather than its total volume is important in influencing renal behavior (6, 40, 41). The great veins (42), the right heart and pulmonary artery (43), the cranial cavity (44), the "cephalad portion of the body" (45), and the arterial pressoreceptors (46) have all been proposed as possible sites for the initiation of afferent impulses designed to modify renal excretion of salt or water. Since the distribution of blood in the vascular tree of a patient with an arteriovenous communication is altered acutely when the shunt is occluded or released, the present study provides additional information by which to evaluate these hypotheses. The data suggest that an increase in arterial pressure or arterial filling may initiate a chain of events culminating in an increased renal excretion of sodium. If this were true, the experimental creation of a large arteriovenous fistula might be expected to promote the renal retention of salt, at least until a new equilibrium is established. This sequence of events has in fact been observed (47, 48).

It is interesting to list the circulatory states, chronic as well as acute, in which the kidneys tend to retain sodium. Dehydration (49), hypoproteinemia (50, 51), and hemorrhage (52, 53) are associated with a diminution in total blood volume. In quiet standing (6), venous congestion of the limbs (54, 55), partial occlusion of the superior or inferior vena cava (56), and portal hypertension (57), filtration from the capillaries is increased and in addition blood is pooled in the peripheral veins, away from the general circulation. Acute (58) and chronic (59, 60) constrictive pericarditis, constriction of the pulmonary artery (61, 62) and congestive heart failure are characterized by distention of the central veins with blood which

the heart is unable to pump efficiently into the aorta. In some of the above conditions the volume of blood in the central veins is reduced; in others these vessels are engorged. In all, however, there exists a tendency toward inadequate filling of the systemic arterial tree, either because of a diminished total blood volume or an altered distribution of blood within the vascular system. The latter may have its origin either in pooling or shunting of blood in the periphery or in failure of the heart as a pump. The changes in sodium excretion, described in the present communication, which accompany occlusion or release of an arteriovenous fistula, are consistent with the hypothesis that renal excretion or retention of sodium is conditioned by the degree of filling of some portion of the arterial tree.

SUMMARY

Occlusion of an established arteriovenous fistula results in an increased renal excretion of sodium. This accompanies the well-known rise in diastolic arterial pressure and slowing of the cardiac rate, despite no change in glomerular filtration rate, renal blood flow or renal venous pressure. The latter values are usually normal and are not altered significantly by surgical repair of the fistula. The data suggest that renal excretion of sodium may be conditioned by the degree of filling of some portion of the arterial tree.

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THE EFFECTS OF QUIET STANDING ON SOLUTE DIURESIS[1]

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INTRODUCTION

In normal supine human subjects (1, 2), the rapid intravenous injection of glucose or mannitol sharply augments the excretion of sodium. Animal experiments indicate that the natriuresis may be mediated at least in large part by a direct renal response to a change in the concentration of the solute in the blood perfusing the kidney (3). It has been suggested that the injected solute restrains the reabsorption of water in the proximal tubules and interferes with the reabsorption of sodium by accelerating the flow of the tubular fluid (1, 3), or by diluting the concentration of tubular sodium below that in extracellular water (4). It is implied in these theories that the capacity of the tubular system for sodium transport is either fixed, or only slowly adaptive to changing loads of sodium. It has therefore been of considerable interest to determine the extent to which, and the rapidity with which, internal regulatory factors may counterbalance the intratubular effects of administered solutes. Certain measures which promote the retention of sodium in normal subjects (antecedent salt restriction, or the administration of DOCA) are comparatively ineffective in preventing the sodium diuresis induced by solute loads (5). However, internal regulatory mechanisms in patients with edema severely limit the excretion of sodium despite massive glycosuria (6) even when the renal hemodynamic pattern is essentially normal. It is not clear, therefore, whether the retention of sodium during osmotic diuresis is a unique feature of the edematous state, or whether the stimuli which have been used to promote salt retention in normal subjects are of insufficient intensity.

Since *quiet standing* constitutes an extremely potent stimulus for sodium and water retention (7–9), the present experiments were undertaken with the following objectives in mind: (*1*) to determine whether normal subjects, under the intense stimulus to retain sodium imposed by quiet standing, nevertheless develop the usual diuresis of sodium with solute loads; (*2*) to decide, by means of clearance techniques, whether the effects observed are mediated by changes in glomerular filtration or tubular transport; and (*3*) to identify, where possible, the changes in the internal environment which may have been conditioning the renal response.

EXPERIMENTAL PROCEDURE

Control and experimental studies were carried out in nine young healthy male subjects. Urine and blood were first obtained while the subject was engaged in normal activity (expts. 1 and 2—Table I), or lying supine on an examining table (expts. 2 to 14—Tables I and II). Next, several samples of blood and urine were collected during *quiet standing* (the subject stood at the side of a shelf on which his arms rested, with only such minimal movement of the trunk and extremities as was found necessary to prevent collapse). Finally, several recovery periods were obtained during which the subject again assumed a supine position. In one experiment (Table II, expt. 9) additional periods during quiet standing and recovery in the supine position followed. Urine was collected in all cases by voluntary voiding. In our experience, the error involved in this method, in selected normal young subjects, is small. The possible effect of such an error on the urinary changes observed between periods of low and those of high flow, has been minimized in most cases in these experiments by averaging the data of more than one period of actual urine collection in each of the "periods" designated *A, B, C, etc.*, in Tables I and II.

Two types of control studies are summarized in Table I. The effects of quiet standing alone, without solute loading, were studied in three experiments during dehydration and hydration. Table I summarizes the effects of injections of hypertonic solutions of sodium bicarbonate and sodium phosphate (pH 7.4) while the subject was supine. Table II summarizes the results of experimental studies of the effects of injections of mannitol and sodium salts during the period of quiet standing.

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Data, in part previously reported by this laboratory (2, 9), on the effects of mannitol loads in supine subjects, and of saline infusions in both supine and standing subjects, have also been used in the construction of Table III and Figures 1 and 2.

In six experiments (expts. 3, 8, 10, 11, 12, 13), glomerular filtration rates were estimated from the clearance of inulin administered as a constant infusion. The isotonic sodium phosphate used in experiments 4 and 14 was prepared as a mixture of NaH_2PO_4 and Na_2HPO_4 of pH 7.4 (10).

Mannitol in serum and urine was analyzed by the method of Smith (11), modified according to Elkinton (12), inulin by the method of Roe (13), potassium and sodium on a flame photometer, and inorganic phosphate by the method of Fiske and Subbarow (14) as modified for the photoelectric colorimeter. Chloride in the serum was determined by the method of Hald (15), protein by the biuret reaction (16), and total CO_2 content by the method of Van Slyke and Neill (17). Urea and ammonia in the urine were determined by the methods of Conway (18), and chloride by a modification of the method of Volhard and Harvey (19). Acute changes in plasma volume were calculated from changes in the hemoglobin and hematocrit as described previously (9). The pH of serum was determined, with anaerobic precautions, with a glass electrode (Beckman). Because of the difficulties of collecting urine anaerobically under the conditions of these experiments, bicarbonate concentrations in the urine were not directly measured. However, since bicarbonate constitutes the major urinary anion in those experiments in which sodium bicarbonate was infused, changes in the excretion of bicarbonate were estimated from changes in the excretion of "undetermined anions" (calculated according to the formula: $A = [Na + K + NH_4] - Cl$, where A is the rate of excretion of "undetermined anions"). In 27 urines collected anaerobically in normal individuals during the infusion of sodium bi-

TABLE I

Control studies. The effects of quiet standing, and of infusions of $NaHCO_3$ and Na_2HPO_4, on the serum and urine of normal subjects

Subject*	Procedure	Period†	Time	Urine							GFR	Serum‡							Change in plasma vol.§
				Flow	Na	Cl	K	NH_4	P‖	An-ions#		Na	Cl	K	CO_2	Prot.	pH	P‖	
		no.	*mins.*	*cc./min.*	*micro. Eq./min.*						*cc./min.*	*mEq./liter*				*gms. %*		*mg. %*	*per cent*
1) AVG (a)	Ambulant	A	140	0.4	44.4	68.0	39.6	26.0				142	101	3.6		7.75			0
	Standing	B	63	0.2	4.7	15.6	27.2	19.5				138	104	3.6		7.95			−12
	Supine	C	131	0.3	6.8	19.1	38.3	21.3				*138*	*106*	*3.7*		*7.05*			+ 5
2) DWS (a)	Ambulant	A	74	0.6	108	125	37.4	16.7	13.3										
	Supine	B	64	0.6	140	100	42.5	13.3	23.5			138	102	3.4	27.5	7.92	7.43	4.5	0
	Standing	C	38	0.4	81.2	92.0	38.6	8.0	16.6			140	102	3.5	27.0	7.25	7.46	4.6	+14
	Supine	D	50	0.2	9.1	12.2	19.0	6.6	11.6			137	102	3.8	24.0	8.18	7.49	3.8	− 6
	Supine	E	69	0.3	18.8	27.0	23.1	8.1	25.8			*142*	*102*	*3.5*	*27.1*	*7.54*	*7.47*	*4.2*	+ 2
3) DWS (b)	Supine	A	66	6.8	294	403	102	41.7	13.5		161	140	105	5.0		6.54		3.5	
	Standing	B	69	1.4	210	270	70.0	37.5	12.7		126	141	105	4.9	25.0	6.78	7.42	3.6	0
	Supine	C	32	1.1	160	230	57.0	38.3	18.7		103	141	105	5.0	22.4	7.22	7.38	3.6	−24
	Supine	D	86	4.7	247	318	78.5	39.9	31.7		*140*	*140*	*105*	*5.0*	*24.2*	*6.20*	*7.48*	*3.6*	+ 4
4) H.,	Supine	A	114	3.1	91.5	83.2	120	16.4	53.4			139	96.0	4.3	26.7	7.12	7.46	4.5	
1 liter, 1%	Supine	B**	116	3.8	161	24.2	210	27.3	472			131	93.0	3.8	25.9	6.71	7.52	10.1	
Na_2HPO_4	Supine	C	91	4.0	248	25.3	203	21.6	584			138	93.3	3.6	26.3	7.00	7.49	10.2	
5) Mac,	Supine	A	108	0.6	79.6	122	34.4	32.8		25									
600 cc., 3.1%	Supine	B**	54	2.6	527	179	191	17.0		556		137	103	4.7	24.4	7.20	7.44		
$NaHCO_3$	Supine	C	146	1.6	371	51.8	151	1.6		471		145	98.4	4.3	31.8	6.28	7.52		
	Supine	D	225	0.8	88.7	29.5	8.4	7.1		74		142	101	4.8	29.1	7.16	7.46		
6) Kat,	Supine	A	66	1.1	172	199	91.5	38.6		104									
550 cc., 2.7%	Supine	B**	104	2.0	377	206	200	11.3		382		140	103	4.3	24.1	7.35	7.43		
$NaHCO_3$	Supine	CW	210	1.2	253	88.5	134	<1		298		144	99.7	3.9	28.0	7.50	7.46		
7) Hal,	Supine	A	98	2.7	200	259	113	26.6		120									
600 cc., 3.1%	Supine	B**	63	6.2	835	330	249	5.5		759		132	95.0	5.7	27.6	7.82	7.30		
$NaHCO_3$	Supine	C	89	2.8	373	127	193	2.1		441		139	94.0	5.4	32.3	6.96	7.32		
	Supine	DW	140	2.2	162	35.8	103	3.4		232		136	93.6	5.1	32.0	7.05	7.40		

* Subjects were normal young adults. AVG, DWS (a), Mac, and Kat were thirsting for 12 to 14 hours before the experiments, and were given no water during the experiments except in period marked w. DWS (b), H, and Hal were allowed water *ad lib.* before the experiments, and drank 100 cc. per hour during the experiments. DWS (b) ingested 15 gms. of NH_4Cl in 36 hours before experiment. This subject also was infused with normal saline containing requisite inulin and sodium para-aminohippurate, at about 2.3 cc. per minute throughout the experiment.

† Infusions of test solutes were given during periods marked**. Water p. o. allowed *ad lib.* in periods marked w. Each period composed of 1 to 3 sub-periods corresponding to individual collections of urine.

‡ Serum values at the beginning of each corresponding period, except for values *in italics* which are at the end of the corresponding period.

§ Changes in plasma volume at the beginning of each corresponding period, calculated with respect to the control period, which is indicated by 0.

‖ P calculated as B_2HPO_4.

See Experimental Procedure for calculation of *anions*.

TABLE II

The infusion of mannitol, $NaHCO_3$, and Na_2HPO_4 into normal subjects during quiet standing

Subject*	Procedure	Period†	Time	Urine								GFR	Serum‡								Change in plasma vol.§
				Flow	Na	Cl	K	NH_4	P‖	Anions#	Mann.		Na	Cl	K	CO_3	Prot.	pH	P	Mann.	
		no.	*mins.*	*cc./min.*	*micro. Eq./min.*						*micro. mol/min.*	*cc./min.*	*mEq./liter*				*gms. %*		*mg. %*	*mM/liter*	*per cent*
8) DWS (c),	Supine	A	138	0.9	207	171	73.4	6.15				112	139	105	4.0		6.68				0
350 cc., 25%	Standing	B**	51	4.9	384	347	77.0	9.43			1,930	110									
mannitol	Standing	C	24	6.8	338	344	65.0	11.6			3,560	123	123	97.1	4.4		6.58			656	+ 5
	Supine	D	87	4.1	215	219	64.6	12.0			1,925	118	*128*	*102*	*4.0*		*6.15*			*92*	+ *8*
9) AVG (b),	Supine	A	53	0.9	133	186	87.0	23.0					145	105	3.9		6.90				0
350 cc., 25%	Standing	B**	29	6.9	320	399	106	31.6			2,255		*137*	*100*	*4.2*		*6.46*			*372*	+*10*
mannitol	Supine	C	35	5.2	293	368	115	18.6			1,700		136	99.2	4.7		7.10			279	− 3
	Standing	D	43	3.7	250	352	120	20.0			1,138		138	104	4.2		6.53			221	+ 7
	Standing	E	60	1.9	52.5	128	57.3	37.2			636										
	Supine	F	51	1.7	90.6	149	67.0	22.2			513		140	105	4.0		7.65			130	−18
10) AVG (c),	Supine	A	123	10.9	172	117	93.7	1.54	49.9			108	140	101	3.9		7.45		3.8		0
1 liter, 4.25%	Standing	B**	80	4.1	105	83.5	29.4	37.4	5.2		787	101	141	100	4.2		7.01		3.2	229	+ 7
mannitol	Supine	C	62	12.7	93.1	56.9	28.3	47.1	18.6		419	102	131	93.3	4.6		7.15		3.6	146	+12
	Supine	D	107	9.6	148	80.0	22.6	30.8	31.7		150	106	*140*	*101*	*4.6*		*6.68*		*4.3*	*16*	+18
11) JCR,	Supine	A	163	1.3	127	160	102	24.7		48		167	*141*	*104*	*4.0*	*25.2*	*6.50*	*7.37*			*0*
525 cc., 3.5%	Standing	B**	47	1.7	329	140	240	9.2		423		195	*142*	*101*	*3.3*	*32.2*	*6.08*	*7.50*			+*11*
$NaHCO_3$	Supine	C	63	1.6	328	67.5	257	1.3		517		141	*140*	*101*	*3.6*	*29.4*	*6.20*	*7.38*			+ *9*
12) AVG (d),	Supine	A	188	5.3	141	179	179	30.5	26.6	171		155									
525 cc., 3.5%	Standing	B	27	7.7	54.6	77.0	85.5	47.5	17.5	112		131	139	103	4.4	27.3	6.82	7.40	2.9		0
$NaHCO_3$	Standing	C**	85	1.3	242	41.5	150	7.2	23.7	358		165	145	102	4.2	31.6	7.16	7.43	3.0		− 6
	Supine	D	113	3.0	395	28.9	159	5.5	59.6	526		170	*142*	*99.4*	*3.9*	*32.0*	*6.07*	*7.43*	*3.2*		+*22*
13) AB,	Supine	A	70	8.6	199	185	73.1	20.8		112		223(?)									
525 cc., 3.5%	Standing	B**	65	3.5	374	204	110	13.0		293		160	140	101	3.9	25.8	6.58	7.35			0
$NaHCO_3$	Supine	C	35	2.9	661	145	193	1.2		710		145	144	96.0	4.3	33.9	6.15	7.50			± 0
14) AVG (e),	Supine	A	147	5.8	67.8	119	146	20.8	53.6				145	102	4.4	28.7	6.77	7.42	3.6		0
1 liter, 1%	Standing	B**	94	3.6	273	22	151	19.0	568				*149*	*96.6*	*3.7*	*28.4*	*6.87*	*7.40*	*17.1*		−*10*
Na_2HPO_4	Supine	C	137	5.7	316	<10	133	15.1	487				*146*	*98.3*	*3.6*	*29.6*	*6.82*	*7.42*	*6.9*		+ *6*

* DWS (c), AVG (b), and JCR were thirsting for 12 to 14 hours before the experiments, and during the experiments, except that JCR drank 250 cc. water 2 hours before the experiment. Other subjects were allowed water *ad lib.* before the experiments, and ingested 100 cc. per hour during the experiments.

See Table I for other footnotes.

carbonate, this estimate has been found to agree with the measured excretion of bicarbonate with an error of 18 ± 9 per cent.

RESULTS

Quiet Standing Alone (Table I):

The effects of quiet standing during hydration and water deprivation were studied in three experiments on two subjects. The passive erect position caused the plasma volume to contract. Urine flow, sodium, potassium and chloride excretion decreased in all experiments, and remained below control values during one or more periods in the supine position. Glomerular filtration decreased in the one experiment in which it was measured. In addition, the excretion of ammonia decreased slightly in the two hydropenic subjects, phosphate excretion was unchanged or declined in two experiments, and the serum bicarbonate decreased slightly, with no significant change in the serum pH in the two experiments in which it was measured.

Quiet Standing with Mannitol Infusion (Table II):

The administration of 350 cc. of 25 per cent mannitol to two thirsting subjects (expts. 8 and 9) prevented any significant contraction of the plasma volume. Presumably the transudation of fluid in the lower extremities caused by quiet standing was balanced by the movement of fluid from the tissues into the blood stream, possibly as a result of two mechanisms: (*a*) the extraction of water from cells (indicated by the decrease in the serum sodium), and its distribution to the plasma volume as a part of the extracellular space; and (*b*) the withdrawal of fluid from the extracellular fluid into the blood, as a result of the transient osmotic gradient set up during diffusion of mannitol from the vascular compartment.

Glomerular filtration was also maintained at control values during the period of quiet standing in experiment 8.

The excretion of water, sodium, and chloride

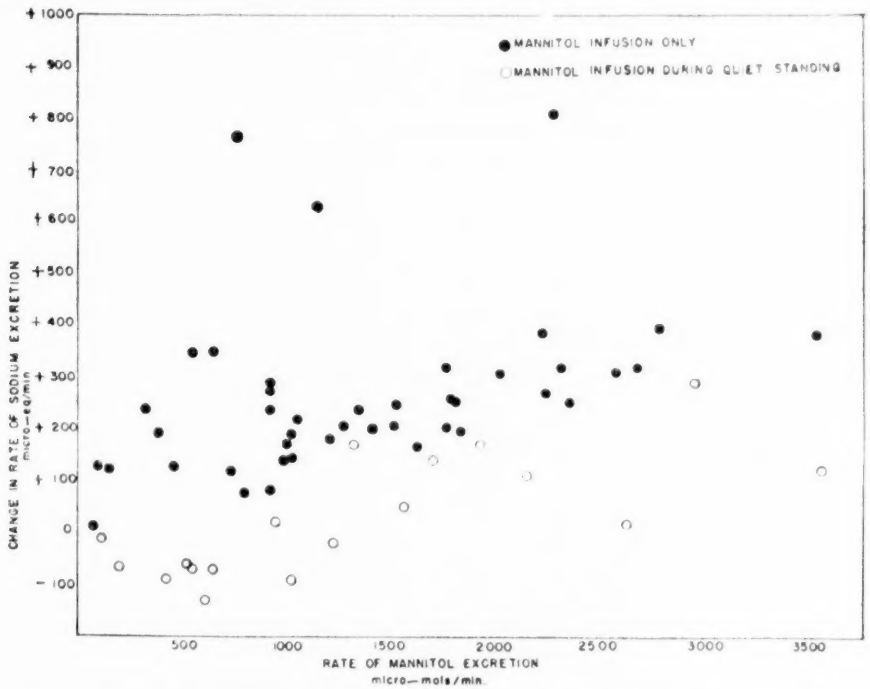

FIG. 1. CHANGES IN THE RATE OF EXCRETION OF SODIUM COMPARED WITH THE RATES OF EXCRETION OF MANNITOL CAUSED BY INFUSIONS OF MANNITOL.

Individual periods of urine collection. Data concerning the infusion of *mannitol only*, taken from seven experiments in part previously reported (2, 20).

TABLE III

Modification of the decrease in sodium excretion during quiet standing by infusions of sodium salts

Infusion given during quiet standing	No. of observations	Mean change* in sodium excretion *micro. Eq./min.*	Range* *micro. Eq./min.*
None**	6	− 91	− 39 to −243
400 cc., 3-4% NaCl**	2	−101	− 30 to −172
400 cc., 6% NaCl**	4	− 41	+273 to −149
500 cc., 3.6% $NaHCO_3$	3	+140	+228 to +117
1,000 cc., isotonic, neutral sodium phosphate	1	+209	—

* Values calculated as the differences between the average rates of 1–3 control periods, and the rates of 1–2 experimental periods.

** Data of 3 of the experiments without infusions, and of all the experiments with NaCl infusions, were taken from another study (4).

increased during the infusion of mannitol and for several periods thereafter (expts. 8 and 9). However, it is evident from Figure 1 that quiet standing in these experiments limited the usual increase in excretion of sodium which is caused in the normal subject by the infusion of mannitol. Indeed, in one of the experiments (expt. 9, Table II), sodium excretion decreased below control values during a second period of quiet standing and then increased again when the subject was supine, although the excretion of mannitol continued slowly to decline.

In a third experiment, on a hydrated subject (expt. 10, Table II), the injection of a smaller quantity of mannitol maintained glomerular filtration at control values, and the plasma volume at or above control values, but sodium excretion declined below control values despite a mannitol diuresis which would have induced marked losses of sodium, if the subject had been supine.

Quiet Standing with the Infusion of $NaHCO_3$ or Na_2HPO_4:

Control Studies: The infusion of 18.75 gms. of $NaHCO_3$ as a 3 per cent solution into normal supine subjects augmented greatly the excretion of water, sodium, and potassium. Chloride ex-

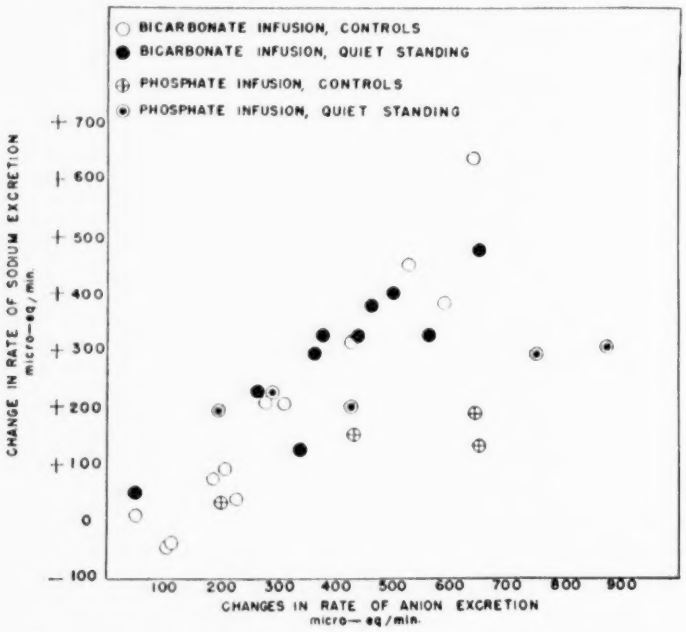

FIG. 2. CHANGES IN THE RATE OF EXCRETION OF SODIUM COMPARED WITH CHANGES IN THE RATE OF EXCRETION OF ANIONS CAUSED BY THE INFUSION OF $NaHCO_3$ AND Na_2HPO_4

Individual periods of urine collection.

cretion initially increased, but then decreased. Ammonia excretion uniformly declined. The concentrations of bicarbonate and sodium in the serum increased; those of chloride and protein decreased.

The infusion of one liter of isotonic Na_2HPO_4 into one normal subject increased greatly the excretion of sodium and potassium, increased slightly the excretion of ammonia, and decreased the excretion of chloride. The concentrations of chloride and protein in the serum decreased slightly, that of phosphate increased greatly.

Studies with Quiet Standing: Injections of sodium bicarbonate and buffered sodium phosphate prevented any significant fall of plasma volume, in three of four experiments (expts. 11, 12, 13), or of glomerular filtration rate in two experiments (expts. 11 and 12). However, sodium excretion was augmented in the same manner during quiet standing as in the supine position in each experiment (Figure 2). This was in sharp contrast to the inhibition of sodium excretion caused by the passive erect posture during mannitol loading (described above) and during salt loading (Table III). In the latter Table, various sodium salts are compared for their effects on the usual retention of sodium induced by quiet standing. Although the excretion of sodium decreased during quiet standing in spite of infusions of sodium chloride (except in one experiment with 6 per cent saline), it increased in all experiments in which sodium bicarbonate or phosphate was infused. Potassium excretion was augmented by the administration of sodium bicarbonate during quiet standing, as in control studies, but was not appreciably changed by infusions of sodium phosphate.

DISCUSSION

A. *Infusions of Mannitol*

In the present experiments on normal subjects during quiet standing, as in previous experiments on patients with edema (6, 20), the sodium diuresis induced by mannitol loads was partially inhibited (Figure 1). Since glomerular filtration rate remained at or above control values (expts. 8 and 10) the inhibition of sodium diuresis appeared to be the result of an increased capacity of the renal tubules to reabsorb sodium. This conclusion is in doubt only to the extent that the estimation of glomerular filtration by present clearance methods is not sufficiently accurate to define the exact relationship between a small (unmeasurable) fall in the filtered sodium and a change in the tubular reabsorption of this ion.

The results do not imply that the renal responses to infused solutes are not mediated at least in part by the direct effect of a change in the solute content and flow of the tubular fluid (3). They do emphasize, however, that internal regulatory mechanisms in normal subjects, as in patients with edema, may be of sufficient intensity to limit the intratubular effects of an administered solute on the reabsorption of sodium. What specific regulatory mechanisms are involved, and whether they may apply to the state of edema, are questions concerning which only inferential data are now available.

In the present experiments, several factors of the internal environment were considered possible stimuli for the retention of sodium:

1. Changes in the serum sodium (Table II), and calculated internal shifts of water and electrolytes were not significantly different during quiet standing from those in previously reported experiments in which mannitol was administered in the supine position (2). In experiments 8 and 9, when hypertonic mannitol was infused, intracellular balances of water and potassium were negative. Positive values for sodium were too small to be considered significant. In experiment 10, when 4.25 per cent mannitol was infused, intracellular balances of water, sodium, and potassium were not significantly altered.

2. Support of the total plasma volume in these experiments by the infusion of mannitol, as in other similar studies by the infusion of albumin (9), failed to eliminate the stimulus for salt retention (Figure 1) elicited by standing. These infusions, however, undoubtedly did not prevent local pooling of blood (in the lower extremities), transudation in the area of increased venous pressure, and consequent redistribution of the flow and volume of the circulating blood. The importance of these factors in the control of salt excretion may be inferred from studies of other abnormal circulatory states in which they may also be operative: Sodium retention occurs with the venous congestion produced by tourniquets on the limbs (21)

or by obstruction of the inferior vena cava (22, 23) and with the portal congestion of decompensated hepatic cirrhosis (24) just as it does when a large volume of blood is actually removed from the circulation by hemorrhage (25). With venous obstruction, cardiac output and arterial blood pressure tend to fall (26). The renal response occurs in spite of lumbodorsal sympathectomy (21) and is independent of the pituitary (27), the adrenals (28) or of measurable circulatory changes in the kidney, but it may be eliminated by very large blood transfusions (26) (which probably overcompensate for blood lost by pooling). The upright position causes vasoconstriction in the cerebral (29) and splanchnic (30) areas; the renal response may occur in the absence of the adrenals (31).

It is possible, therefore, that in the present experiments, although there was no hemoconcentration when mannitol was infused during quiet standing, the kidneys may have responded not to the circulating blood volume as a whole, but to a change in the flow and/or volume of blood in some "central" area of the circulation (32, 33).

B. *Infusions of Sodium Bicarbonate and Phosphate*

In order to determine more precisely the intensity and character of the increased tubular capacity for sodium transport which is induced by quiet standing, sodium bicarbonate and buffered sodium phosphate were injected in experiments 11 to 14. These salts impose unique limitations on the tubular reabsorption of sodium. When the anion of an administered electrolyte is reabsorbed only to a slight extent, the total excretion of cations into the urine is correspondingly increased because of the limited quantity of free acid which may be secreted by the tubules.[4] The factors which determine the nature of the "covering" cation are unclear, but under ordinary conditions, particularly when the administered electrolyte is a sodium salt, this cation is mostly sodium. In the presence of a strong stimulus for the retention of sodium, it might be expected that the increased cation would be largely potassium (or conceivably partly ammonium, in the case of phosphate or sulfate). Indeed, when sodium bicarbonate is administered to patients with congestive heart failure or with "decompensated" hepatic cirrhosis, the bicarbonate ion is excreted to a great extent with potassium (20). However, in the present experiments, injected sodium bicarbonate and sodium phosphate were excreted much more easily during quiet standing than were similar quantities of sodium chloride which were of about the same tonicity and which increased the serum sodium to approximately the same extent (Table III). Furthermore, standing did not alter the role of sodium as the chief "covering" cation in the urine (Figure 2). The increased excretion of potassium was similar to that which was observed in the absence of a stimulus for the retention of sodium.

Two explanations for these results suggest themselves: (*1*) the effects of administered phosphate or bicarbonate on the excretion of sodium involve renal tubular mechanisms which may be entirely distinct in location (proximal *vs.* distal) or character (ion exchange *vs.* trace resorption) from those involved in the sodium retention of quiet standing; or (*2*) the stimulus for sodium retention imposed by quiet standing is not sufficiently powerful to counteract the unique limitation imposed by the excretion of bicarbonate and phosphate on the reabsorption of sodium. It may be that the excretion of sodium bicarbonate would be curtailed in the normal subject, as it is in the patient with edema (20, 34–36), if the stimulus for the retention of sodium were intense enough and sufficiently prolonged.

[4] This limitation is imposed not only because of the extreme acidity of acids such as H_3PO_4 and H_2SO_4, but also, in the case of bicarbonate, because of the probability that CO_2 has a great capacity to diffuse back rapidly across the tubular membrane.

SUMMARY

Infusions of mannitol, sodium bicarbonate and sodium phosphate were administered to normal subjects in the supine position and during quiet standing.

Observations were made of changes in the plasma volume, the serum electrolytes, and the urinary excretion of the loading solute, and of sodium and other electrolytes.

It was found that quiet standing partially inhibited the sodium diuresis induced by the administration of mannitol, but did not interfere with the increased excretion of sodium caused by infusions of $NaHCO_3$ or Na_2HPO_4.

The results with mannitol indicate the operation of internal regulatory mechanisms on the intrinsic renal responses which characterize solute diuresis.

The results with sodium bicarbonate and phosphate are discussed in relation to the unique limitations imposed by these salts on the tubular reabsorption of sodium.

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THE GLYCOGEN CONTENT OF HUMAN LEUKOCYTES IN HEALTH AND IN VARIOUS DISEASE STATES [1]

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INTRODUCTION

Studies by Wagner (1) on the quantitative estimation of glycogen in human blood disclosed the average glycogen content per 100 ml. to be 5.5 mg. In separated leukocytes an average of 2.54 micrograms of glycogen was found per 10^6 total leukocytes, or 4.23 micrograms per 10^6 granulocytic leukocytes. No detectable glycogen is present in human plasma or erythrocytes. It has been both denied (2) and claimed (3) on the basis of histochemical data that lymphocytes and platelets contain small amounts of glycogen. The work of Wagner (1, 4), however, would indicate that the granulocytic leukocyte is probably the only significant representative of the white cells in the peripheral blood which contains glycogen. Studies on leukemic leukocytes (4) indicate that the blast cell contains no measurable amount of glycogen. Both histochemical studies (5) and the quantitative observations of Wagner (1, 4) suggest that glycogen first appears in the granulocytic leukocytes at the myelocytic stage and may increase with increasing cell maturity. Wagner (4) has observed a normal content of glycogen per 10^6 leukocytes in chronic myelocytic leukemia. In this laboratory, recent studies (6) have shown that whereas total leukocyte glycogen is frequently within the normal range, *unit myeloid cell leukocyte glycogen* is low. This is not explainable solely in terms of cell immaturity in the leukemic blood analyzed. In polycythemia vera of the type associated with leukocytosis and leukemoid reactions, the unit myeloid cell glycogen is substantially above normal (6). Wagner (4) has observed increased leukocyte glycogen values in polycythemia vera and Wachstein (5) has made similar qualitative observations on the basis of histochemical staining techniques. The leukocyte glycogen content may be remarkably increased in Von Gierke's disease (1, 7).

It is the object of this study to present further observations on the glycogen content of human leukocytes in health and in various disease states employing an adaptation of the anthrone technique (8, 9) to glycogen measurements in white blood cells.

METHODS

Since the myeloid leukocytes are apparently the only significant source of blood glycogen (1) the following method has been employed in the estimation of leukocyte glycogen. Blood was collected as for the phosphatase determination (10) in a freshly prepared saline solution of bovine fibrinogen (Fraction I), Armour, and the erythrocytes allowed to settle. Since this process is relatively rapid and satisfactory separation nearly invariable by thirty minutes, it was possible within this space of time to remove the practically red-cell-free but white-cell-rich supernatant to a separate container. This leukocyte-platelet suspension was thoroughly mixed and 1 ml. aliquots pipetted into triplicate boiling tubes containing NaOH in final concentration of 30 per cent. Total leukocyte counts were made in triplicate on the mixed suspension subjected to analysis, and in duplicate on a simultaneously removed specimen of venous blood. Blood smears were also obtained from the ear at the time of venepuncture and 200 cell differential leukocyte counts made. The remaining leukocyte suspension was then centrifuged and 1 ml. of the *cell-free supernatant* pipetted into each of two tubes containing NaOH in the same manner as the unknowns. These served as (*1*) a blank and (*2*) a standard after addition of a known amount of glucose since their composition was identical with that of the unknown except for the contributions of the cells. All material was digested by boiling for 45 min. in a water bath, the tubes being covered with glass marbles to prevent evaporation. At the end of 45 min. sufficient 95 per cent alcohol was added to each tube to bring the final alcohol concentration to 60 per cent. The glycogen which precipitates at this concentration of alcohol was allowed to settle and the precipitate centrifuged. The precipitate was then washed twice with 60 per cent alcohol and, after removal of the bulk of the final supernatant, dried at approximately 100° C in a drying oven. Each of the triplicate unknowns was suspended in 4 ml. of distilled water and to this was added

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TABLE I

Glycogen content of separated leukocytes in normal subjects

Subject	W.B.C. per cu. mm.	Glycogen content		
		Per 10^{10} leukocytes mg.	Per 10^{10} myeloid leukocytes mg.	Per leukocytes of 100 ml. of blood mg.
A. L.	5,250	40.9	66.7	2.13
H. F.	6,250	60.5	118.6	3.78
C. E.	4,850	39.9	79.8	1.93
A. L.	8,700	43.2	69.2	3.75
A. S.	8,150	50.2	85.0	4.08
E. C.	6,075	48.8	76.3	2.96
R. H.	7,750	42.0	81.5	3.24
V. H.	9,375	66.0	85.0	6.20
C. C.	8,625	60.0	93.8	5.17
M. H.	7,425	41.6	73.8	3.08
H. F.	6,325	31.8	53.0	2.02
J. O.	8,075	49.2	65.2	3.97
G. S.	10,300	57.0	79.3	5.86
E. A.	6,350	34.8	51.2	2.21
J. B.	8,250	44.7	70.0	3.69
A. A.	8,650	62.4	99.0	5.37
H. K.	7,125	74.0	86.0	5.27
F. C.	6,450	43.0	66.2	2.77
N. W.	6,400	38.0	47.2	2.43
H. H.	10,250	35.7	49.0	3.68
M. L.	7,325	48.6	81.0	3.56
Mean:		48.2	75.1 ± 17.4*	3.61
Range:		31.8–74.0	47.2–118.6	1.93–6.2

* Standard deviation.

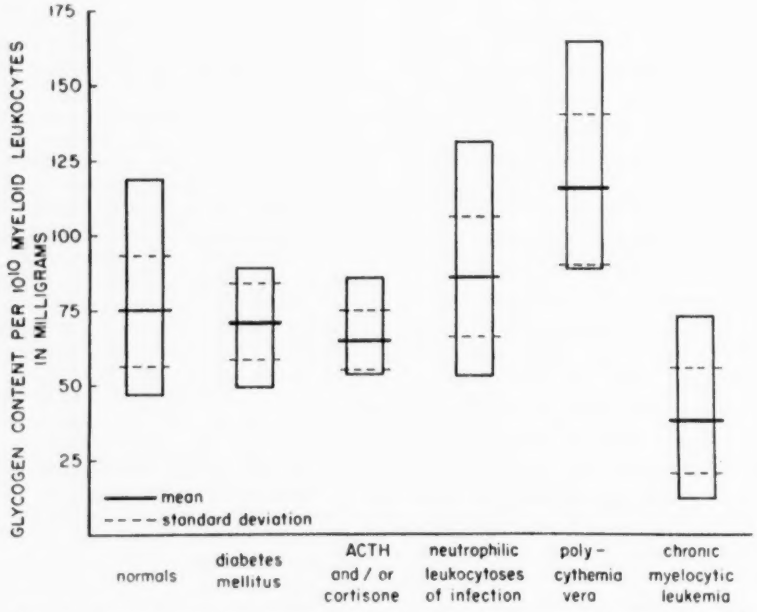

FIG. 1. MEAN, STANDARD DEVIATION, AND RANGE OF GLYCOGEN CONTENT PER 10^{10} MYELOID LEUKOCYTES

The data in diabetes mellitus have excluded a single, unexplainably high value as discussed in the text. The data for polycythemia vera refer *only* to cases of this syndrome with concomitant leukocytosis and/or leukemoid reaction.

8 ml. of the anthrone reagent (8, 9) (0.2 per cent anthrone in concentrated H_2SO_4). The blank was identically treated and the standard was prepared by adding 4 ml. of distilled water containing 50 micrograms of glucose to the second blank material. The color developed was read after 10 minutes at wave length 620 using the Beckman Model B spectrophotometer. The determination was related to the glucose standard and the glycogen content of the unknowns computed by the standard formula:

$$\text{micrograms of glycogen in aliquot} = \frac{50 \times V}{1.11 \times S}$$

V—optical density of unknown,

S—optical density of 50 microgram glucose standard,

1.11—factor of conversion of glucose to glycogen.

To our knowledge the anthrone method (9) has not previously been employed specifically for leukocyte glycogen. It has the advantage that hydrolysis of the purified glycogen is not necessary. The anthrone reagent must be prepared fresh, the sulphuric acid of high purity, and care must be taken to prevent contamination with lint or other material which will react with this reagent.

RESULTS

Normal subjects. Table I and Figure 1 show the leukocyte glycogen values in 21 normal subjects. The latter were ostensibly healthy individuals admitted to the hospital for such conditions as hernia repair and all samples were analyzed prior to any corrective procedures. All had total and differential leukocyte counts within the normal range. By the anthrone technique mean leukocyte glycogen per 10^{10} total leukocytes was 48.2 mg. with a range of 31.8 to 74.0 mg. The more significant mean value of glycogen content per 10^{10} myeloid cells (since the only significant leukocyte carrier of glycogen appears to be the granulocytic cells) was 75.1 mg. and the range 47.2 to 118.6 mg. The mean glycogen content of the leukocytes separated from 100 ml. of blood was 3.61 mg. and the range 1.93 to 6.20 mg. These values for separated leukocytes are somewhat higher than those of Wagner (1) but more closely approximate the theoretical expectancy on the basis of whole blood glycogen values. Wagner explained his less than expected values in terms of glycogen degradation during the separation procedure. The difference in separation technique and the short interval between the time of blood withdrawal and the introduction of leukocytes into the alkali digestion mixture may explain the higher values here obtained.

TABLE II

Effect of fasting and food ingestion on leukocyte glycogen

Subject	Glycogen per 10^{10} leukocyte mg.	Remarks
J. C.	157.0*	Fasting
	149.5	105 min. p.c.
H. L.	53.0	Fasting
	46.3	105 min. p.c.
D. H.	71.6	Fasting
	68.9	105 min. p.c.
B. F.	67.2	Fasting
	73.1	90 min. p.c.
R. P.	43.2	Fasting
	51.0	75 min. p.c.

* Polycythemia vera with leukocytosis responsible for high values.

Effect of fasting and food intake on leukocyte glycogen. Table II shows a comparison of leukocyte glycogen values determined on the same day during the fasting state and 1¼ to 1¾ hours after a routine breakfast in subjects with various diseases. It can be readily seen that the postprandial values are essentially the same as those obtained before ingestion of food. It has, therefore, not been deemed necessary to employ fasting blood only in these determinations.

Diabetes mellitus. Table III summarizes the findings of 16 determinations on 15 diabetic subjects. All but two were receiving insulin. In twelve instances the studies were performed on fasting blood on which blood sugar and carbon-dioxide combining power determinations were simultaneously made. In all subjects the diabetes was insufficiently controlled in terms of fasting blood sugar values and/or urinary sugar. In the 12 instances in which simultaneous blood sugar determinations were made they were found to range from 138 to 608 mg. per cent. One subject, J. J., was in coma. The blood sugar was 608 mg. per cent and the carbon-dioxide combining power less than 5 volumes per cent. Despite the diabetic state and high blood sugar levels at the time of the studies, with one exception the mean glycogen per 10^{10} myeloid leukocytes was within the normal range. The mean for the entire group was only slightly higher than normal and this could be attributed entirely to the unexplainably high value obtained in a single patient (J. R.). In Figure 1

TABLE III

Glycogen content of leukocytes in poorly controlled diabetes mellitus

Subject	Glycogen content: Per 10^{10} leukocytes mg.	Glycogen content: Per 10^{10} myeloid leukocytes mg.	Glycogen content: Per leukocytes of 100 ml. of blood mg.	Insulin	Blood sugar mg. %
G. S.*	47.7	82.2	1.85	yes	
A. P.*	43.4	61.4	1.84	yes	
W. S.*	80.1	85.0	24.83	yes	
P. I.*	50.0	65.0	5.10	yes	
I. I.†	54.1	64.0	14.00	no	608
P. I.	48.6	77.0	4.34	yes	228
J. R.	117.5	163.0	10.20	yes	185
J. D.	57.7	88.7	4.55	yes	460
J. B.	42.7	88.4	3.12	yes	150
L. S.	62.9	69.5	8.70	yes	330
J. H.	38.8	76.8	2.32	no	190
A. M.	37.8	49.7	3.04	yes	214
S. L.	30.0	58.9	1.29	yes	200
C. S.	37.3	54.8	2.56	yes	138
J. C.	68.3	84.7	7.13	yes	276
H. C.	56.8	82.3	3.18	no	190
Mean:	54.6	78.5 ± 26.9‡	6.13		
Range:	30.0–117.5	49.7–168.0	1.29–24.83		

* In poor control but blood sugars not measured at time of glycogen determination.
† In coma. CO_2 combining power of blood less than 5 volumes per cent. All blood sugar determinations made on sample of blood analyzed for glycogen.
‡ Standard deviation. Large standard deviation is chiefly result of inclusion of unexplainably high value obtained on patient J. R.

the range and mean glycogen values per 10^{10} myeloid cells are shown *with the exclusion of patient J. R.* It appears that neither the metabolic defect of diabetes mellitus nor the high sugar environment of the leukocytes in these subjects is reflected in any deviation of leukocyte glycogen from the normal range.

Subjects receiving cortisone and/or ACTH. ACTH and cortisone therapy are well known to have profound effects on carbohydrate metabolism and glyconeogenesis. It was, therefore, considered of interest to investigate the glycogen content of separated leukocytes in subjects receiving large doses of these agents. Table IV and Figure 1 show the findings in nine such subjects. Seven subjects were receiving cortisone alone in doses ranging from 75 to 200 mg. a day. One subject (L. H.) was receiving 150 mg. of cortisone a week

TABLE IV

Glycogen content of leukocytes in subjects receiving cortisone or ACTH

Subject	Glycogen content: Per 10^{10} leukocytes mg.	Glycogen content: Per 10^{10} myeloid leukocytes mg.	Glycogen content: Per leukocytes of 100 ml. of blood mg.	Therapy
C. H.	38.9	53.2	2.06	ACTH 75 mg. daily and cortisone 75 mg. daily
W. T.	54.2	70.4	8.33	Cortisone 75 mg. daily
S. L.	60.0	86.3	7.06	Cortisone 75 mg. daily
P. Y.	43.5	60.0	5.22	Cortisone 75 mg. daily
F. N.	46.8	73.8	5.33	Cortisone 100 mg. daily
L. H.	39.4	54.3	3.22	Cortisone 150 mg./week
A. D.	51.8	62.8	7.06	Cortisone 200 mg. daily
O. C.	59.4	68.7	8.80	Cortisone 150 mg. daily
P. C.	45.0	56.5	6.36	Cortisone 150 mg. daily
Mean:	48.8	65.1 ± 10.8*	5.94	
Range:	38.9–60.0	53.2–86.3	2.06–8.80	

* Standard deviation.

and one (C. H.) was receiving 75 mg. of ACTH and 75 mg. of cortisone daily. The underlying disease process in these patients included atopic dermatitis, rheumatoid arthritis, and scleroderma. Although the mean glycogen content per 10^{10} myeloid leukocytes was somewhat lower than that of the larger group of normal subjects, all values were well within the normal range. Only one value was above the normal mean.

Neutrophilic leukocytosis of infection. Twenty-eight determinations of leukocyte glycogen content were made in 24 subjects with neutrophilic leukocytosis of infection. In two determinations recorded, the leukocyte count was within the normal range but one day previously had been elevated on the basis of an infectious process. The subjects studied had pneumonia, tonsillitis, meningitis, urinary tract infection, surgical complications, acute appendicitis, tuberculous pericarditis, and bacteremia of various types as a cause of leukocytosis. The mean glycogen content per 10^{10} myeloid leukocytes was 86.7 mg., substantially above the mean for normal subjects. Comparison with other disease states and with normal subjects is indicated in Figure 1. Twenty-one of twenty-eight determinations were above the normal mean but only two above the highest normal value obtained. However, almost 40 per cent of the values were above 90 mg. while only 14 per cent of values in normal subjects were in this range. It may be concluded that the glycogen content of myeloid leukocytes is appreciably elevated on the average in presence of neutrophilic leukocytosis of infection. These elevated leukocyte counts and high unit cell glycogen values, of course, are reflected in marked elevations in total circulating leukocyte glycogen.

Determinations were also made in eight cases with neutrophilic leukocytosis associated with such non-infectious processes as decompensated liver disease and metastatic carcinoma. All values per 10^{10} myeloid leukocytes were within the normal range and only one above 88 mg. This subject had metastatic carcinoma and marked leukocytosis ranging from 60,000 to 78,000 per cu. mm. On successive determinations unit cell glycogen was well above that of the highest normal subject, being over 130 mg. per 10^{10} myeloid leukocytes. In this subject the leukocyte glycogen content per 100 ml. of blood was as high as 102 mg., more than 14 times the highest value obtained in any normal subject.

Chronic myelocytic leukemia, polycythemia vera with leukocytosis and/or leukemoid reactions, and other "myeloproliferative syndromes." The data obtained on leukocyte glycogen in these conditions has been incorporated in a separate study (6) reported elsewhere and will only be summarized here. In chronic myelocytic leukemia, while the glycogen content per 10^{10} leukocytes of all types is usually within the normal range, the more significant value per 10^{10} myeloid leukocytes was consistently low in 14 subjects with this disease. The mean value was 37.9 mg., approximately one-half that of normal subjects. In sharp contrast, myeloid leukocyte glycogen in polycythemia vera of the type associated with marked leukocytosis and leukocyte immaturity was higher than in any other disease state studied. The mean content per 10^{10} myeloid leukocytes was 116.2 mg. in 15 subjects. The differences are not explainable in terms of therapy or the differential leukocyte picture and must be considered as evidence of a marked metabolic difference in the two conditions. This is true even in cases which clinically may be suspected of having undergone so-called leukemic transition. In other "myeloproliferative syndromes" (11) thought not to be either polycythemia vera or chronic myelocytic leukemia, but with similar leukocyte pictures, the pattern of glycogen content has not been consistent. Some have exhibited high glycogen content of myeloid leukocytes; others have shown low or normal values. These findings have been discussed previously in more detail (6). For purposes of comparison, the values in chronic myelocytic leukemia and in polycythemia vera are incorporated in Figure 1.

Miscellaneous disease states. In acute blastic leukemias and in chronic lymphocytic leukemias the glycogen content per 10^{10} leukocytes of all types was very low. Since in most instances some cells of the myeloid series were also present, it cannot be categorically stated that blast cells and lymphocytes are completely glycogen-free. In a single instance of chronic lymphocytic leukemia in which a 200 cell differential leukocyte count revealed 100 per cent of the cells to be lymphocytes, no measurable glycogen was found, however. Moreover, in two cases of infectious mononucleosis in which mononuclear cells constituted a higher than normal per-

centage of the total leukocytes, low glycogen values per 10^{10} leukocytes were also observed. It may be concluded that the data support the concept that myeloid cells beyond the blast stage of maturity are the only significant carriers of glycogen among the circulating leukocytes.

DISCUSSION

It can be presumed that the glycogen within the myeloid leukocytes serves as a storehouse of reserve energy conveniently available to meet metabolic needs in carrying out normal physiological functions. The leukocytes of the blood are freely floating cells in fluid environment containing glucose readily available to the entire cell surface. It is of interest that the glycogen content remains relatively unchanged during the immediate postprandial period when blood glucose is known to rise and that it is also within the normal range in poorly controlled diabetic subjects with marked elevation in blood sugar and the metabolic handicap of the diabetic state. Likewise, leukocyte glycogen does not appear to be particularly sensitive to cortisone and ACTH therapy, although the mean value per 10^{10} myeloid leukocytes in the relatively small group of patients receiving these medications was somewhat below the normal mean.

On the other hand, the low glycogen content of myeloid leukocytes in chronic myelocytic leukemia appears to be a consistent metabolic aberration in this disease while in polycythemia vera of the type with associated leukocytosis and/or leukemoid reaction, even when the blood picture is indistinguishable from chronic myelocytic leukemia, very high glycogen values are consistently observed. Dempsey and Wislocki (12) have commented upon the possible interrelationship of alkaline phosphatase and glycogen within cells. Observations in this laboratory (6, 10, 13) have indicated a certain parallelism between glycogen content and alkaline phosphatase activity of leukocytes. In conditions where blast cells or lymphocytes are the predominating cell type, both glycogen content and alkaline phosphatase activity of leukocytes are low. In chronic myelocytic leukemia a similar situation exists whereas in polycythemia vera with leukocytosis and/or leukemoid features, both values tend to be well above the normal range. The parallelism is not absolute, however. On the average, the highest unit cell leukocyte alkaline phosphatase values are found in the neutrophilic leukocytoses of infection. Glycogen content is also elevated above normal in this situation, but not quite so strikingly as in polycythemia vera with leukocyte abnormalities where the highest values are observed. In this connection, it should be mentioned that in polycythemia without concomitant leukocyte abnormalities, both unit leukocyte alkaline phosphatase and glycogen are usually normal. Whether this parallelism between glycogen content and alkaline phosphatase activity is fortuitous or represents a metabolic interrelationship cannot be stated with certainty.

SUMMARY

Data are presented on the glycogen content of separated human leukocytes in health and in various disease states.

Leukocyte glycogen has been observed to remain relatively unchanged during the postprandial rise in blood sugar, in poorly controlled diabetes mellitus and in the presence of massive cortisone therapy.

Unit cell myeloid leukocyte glycogen tends to be substantially low in chronic myelocytic leukemia and high in polycythemia vera with leukocytosis and/or leukemoid features. It also tends to be substantially above normal in the neutrophilic leukocytoses of infection.

The data confirm previous observations that lymphocytes and blast cells are either glycogen-free or extremely poor in glycogen content.

The parallelism between unit leukocyte glycogen and alkaline phosphatase activity is discussed.

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METABOLIC AND RENAL STUDIES IN CHRONIC POTASSIUM DEPLETION RESULTING FROM OVERUSE OF LAXATIVES [1, 2]

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INTRODUCTION

It is well known that diarrhea caused by a variety of gastrointestinal disorders may lead to potassium depletion (1). This paper presents observations on two otherwise healthy women who, prior to this study, had gradually developed severe potassium depletion and hypokaliemia as the result of chronic diarrhea induced by overuse of laxatives. Although in each instance there had been a loss of approximately one-third of total normal body potassium, there were no other significant disturbances of water and electrolyte balance and no overt signs of malnutrition. These two subjects thus presented an unusual opportunity to observe the clinical and physiological effects of uncomplicated potassium depletion.

The case histories described below present the clinical observations made before and after the spontaneous restoration of potassium balance which occurred when laxatives were withheld and the patients given a normal diet.

This paper reports the results of balance studies carried out in both subjects for the three-week period in which repair of their potassium deficit was accomplished. In addition, various renal functions were measured serially. In one patient renal function studies were begun immediately after the potassium deficit had been repaired, but in the second patient measurements were made both before and after treatment. The electrocardiographic findings in these patients will be discussed elsewhere (2).

CASE REPORTS

Case 1

R. S., a 24 year old woman, was referred for investigation of her chronic complaints of vague muscle pains, malaise, and weakness. Although her appetite was usually good and she had not lost weight, she stated that under emotional stress she often ate little or nothing for a day or two at a time. For more than a year she had observed a tendency to gain weight and to develop ankle edema whenever she ate salt. Up to the day of admission she had worked regularly as an IBM machine operator.

Physical examination revealed a somewhat thin, but otherwise healthy-appearing female. There were no abnormal findings. Muscle strength and tendon reflexes were normal.

Laboratory studies of the blood, stool and urine were not remarkable except that the maximum specific gravity of the urine after 18 hours of dehydration was 1.013. X-ray examinations of the chest, long bones, skull, kidneys, and entire gastrointestinal tract revealed no abnormalities. Sigmoidoscopic examination was negative. A routine electrocardiogram revealed flattened or diphasic T-waves in most leads and was thought to be suggestive of hypokaliemia. Repeated determinations of serum potassium concentrations gave values between 2.1 and 2.3 mEq. per l. Serum bicarbonate concentration was 30 to 33.5 mEq. per l., but analyses for the following were normal: serum sodium, chloride, albumin, globulin, cholesterol, calcium, phosphorus, and blood sugar and urea nitrogen. A standard 8 lead electroencephalographic record was within normal limits.

During the next three weeks, while on balance study, she spontaneously corrected her potassium deficit and her electrocardiogram returned to normal. At this time she first admitted to the habitual ingestion of Hinkle's Cascara Compound [4] which she had taken in quantities of 15 to 30 tablets per week in order to produce 2 to 3 watery bowel movements every day. At the completion of the period of potassium retention she was given laxatives for

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[2] Presented in part in abstract at the 44th Annual Meeting of the American Society for Clinical Investigation, May 1952.

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[4] Each tablet contained: .016 gm. cascara, .016 gm. aloin, .01 gm. podophyllum, .008 gm. extract of belladonna, .001 gm. strychnine sulfate, and .004 gm. oleoresin of ginger.

a few days to observe the effect on potassium balance and the electrocardiogram. During the next six months the patient was free of diarrhea and her serum potassium concentration and electrocardiogram remained normal.

Case 2

A. B., a 42 year old widow, came to the hospital complaining of vague malaise, weakness and abdominal discomfort. She had been accustomed for a long time to taking indeterminate but large amounts of proprietary laxative compounds containing phenolphthalein, podophyllum, and aloin. These produced several watery bowel movements each day. Her appetite and intake of food were usually normal, but occasionally, at times of emotional stress, she ate little or nothing for several days at a time. For several months she had noted that her ankles swelled intermittently. Prior to admission she was able to work regularly at her job as a saleslady.

On physical examination the patient looked depressed and chronically ill. There were no other abnormal findings. Tendon reflexes were normal and her muscle strength seemed appropriate.

Routine laboratory studies of the blood, stool and urine were not remarkable except that maximal specific gravity of the urine after 18 hours of dehydration was 1.005. X-ray examinations of the long bones, chest, stomach, small intestines, and gall bladder revealed nothing of significance. A barium enema showed a moderately atonic, redundant colon, but sigmoidoscopic examination disclosed no evidence of disease.

A routine electrocardiogram showed sagging of the ST segments and prominent U-waves in most leads. Because of this suggestive evidence of hypokaliemia, serum potassium analyses were done. Repeated analyses gave values between 1.6 and 2.3 mEq. per l. Serum chloride concentration was 94 mEq. per l. but analyses for the following were normal: serum sodium, carbon dioxide content, albumin, globulin, cholesterol, calcium, phosphorus, blood sugar, and urea nitrogen. A standard 8 lead electroencephalogram was within normal limits.

The patient was placed on balance study, and during the next three weeks her potassium deficit was corrected and the electrocardiogram returned gradually to normal. During this time she noted a gradual, definite improvement in vigor and appetite and in her sense of well-being. Following discharge, the concentration of serum potassium, determined at frequent intervals, was usually normal, as were electrocardiographic records made at the same time. On two occasions associated with emotional stress she suffered brief episodes of anorexia and diarrhea, which she said were not provoked by laxatives. At these times the serum potassium concentration was low and the electrocardiogram showed T-wave abnormalities.

METHODS

The patients were allowed to be ambulatory on the ward. Diarrhea had stopped or diminished prior to the beginning of the balance study and after a few days all bowel movements were of normal frequency and consistency. The patients were given constant weighed diets which contained little sodium, a low-normal amount of potassium and the approximate nitrogen and caloric content of the usual ward diet. No food was refused during the period of study. Salt was added each day in fixed amount with a weighed salt shaker in order to raise the sodium intake to a normal level. Potassium chloride, 20 to 40 mEq. per day, was added to the oral intake as indicated in Tables I and II, but at no time did the total potassium intake exceed the usual range of normal. No other medications were administered.

Organic acids in the urine were determined by the method of Van Slyke and Palmer (3) adapted for use with the Beckman pH-meter. No creatinine correction was applied. Analyses for calcium in diet, urine and stool were carried out by dry-ashing, followed by a modification of the Tisdall and Kramer titration (4). Total exchangeable potassium was determined[5] by analysis of urine specific activity 24 hours after the administration of an oral tracer dose of $K^{42}Cl$ (5). The chemical methods used to determine electrolytes in red blood cells will be published elsewhere (6).[6] The other analytical procedures, the methods used in the calculation of results, as well as other details of the balance technique have been described in a previous paper (7).

The measurements of inulin clearance (C_{In}), p-aminohippurate clearance (C_{PAH}), endogenous creatinine clearance (C_{Cr}), and the maximal tubular secretory rate for PAH (Tm_{PAH}) were made in the standard manner (8) with the patients well-hydrated and resting in the supine position for at least an hour before the beginning of each study. On one occasion the renal arteriovenous oxygen difference (A-V O_2) and the renal extraction of PAH (E_{PAH}) were determined in patient A. B. by the use of simultaneous samples obtained from an inlying peripheral arterial needle and a catheter placed in the right renal vein.[7] In this case, total renal blood flow was calculated from C_{PAH}, E_{PAH}, and the peripheral venous hematocrit (Hkt). Renal oxygen consumption (R O_2) was estimated as the product of total renal blood flow and A-V O_2.

The analyses for inulin in serum and urine were done by the method of Roe (9); PAH was determined by Smith's adaptation (10) of the Bratton and Marshall diazo reaction (11), and endogenous creatinine was measured by Hare's modification (12) of Borsook's method (13), which employs Lloyd's reagent for the separation of creatinine from non-creatinine chromogens.

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[7] The authors are indebted to Drs. Walter E. Judson and James D. Hatcher, Evans Memorial, Massachusetts Memorial Hospitals, for their assistance with this procedure.

TABLE I—

A. B. 9 42		Intake							Urine						
Day	Body weight Kgm.	H_2O ml.	Na mEq.	K mEq.	Cl mEq.	N gms.	P gms.	Ca mgs.	Vol. ml.	Na mEq. per day	K mEq. per day	Cl mEq. per day	P gms. per day	NH_4 mEq. per day	N gms. per day
1	44.52	1,200	92	44	94	8.4	.56	100	981	11	9	23	.46	73	7.6
2	44.56	2,000	92	44	94	8.4	.56	100	890	11	8	22	.25	51	6.7
3	45.09	2,000	92	44	94	8.4	.56	100	1,160	17	7	23	.28	49	7.5
4	45.42	2,000	92	44	94	8.4	.56	100	1,370	18	8	20	.34	49	6.9
5	45.61	2,900	92	64	114	8.4	.56	100	1,110	22	9	26	.34	43	6.7
6	45.82	2,020	92	84	134	8.4	.56	100	1,060	27	9	34	.25	36	5.8
7	46.42	2,010	92	64	114	8.4	.56	100	1,019	27	10	32	.25	31	5.5
8	46.79	2,020	92	84	134	8.4	.56	100	1,130	37	12	44	.34	34	5.8
9	46.99	2,020	92	84	134	8.4	.56	100	1,210	45	16	56	.37	39	5.7
10	47.10	2,020	92	84	134	8.4	.56	100	1,480	73	23	89	.37	38	6.1
11	47.69	2,020	92	84	134	8.4	.56	100	1,660	80	24	90	.37	30	7.5
12	47.96	2,020	92	84	134	8.4	.56	100	1,780	128	36	143	.34	27	6.8
13	47.73	2,020	92	84	134	8.4	.56	100	1,805	152	42	165	.34	23	7.6
14	47.75	2,020	92	84	134	8.4	.56	100	3,310	313	73	328	.34	18	7.0
15	46.05	2,020	92	84	134	8.4	.56	100	2,230	168	56	191	.53	15	5.1
16	45.43	2,020	92	84	134	8.4	.56	100	2,410	194	90	224	.53	25	7.2
17	44.97	2,020	92	84	134	8.4	.56	100	2,320	152	84	181	.47	12	6.5
18	44.32	2,020	92	84	134	8.4	.56	100	1,520	77	64	107	.53	20	6.8
19	44.34	2,020	92	84	134	8.4	.56	100	1,630	66	81	108	.59	22	7.3
20	44.69														

Analyses of oxygen content of whole blood were made according to Van Slyke and Neill (14).

Renal concentrating ability was tested by determining the maximum urinary specific gravity (measured by a calibrated urinometer or by direct weighing of urine on an analytical balance) after 18 to 24 hours of dehydration.

RESULTS

I. *Metabolic Studies*

A. *Potassium balance and serum potassium*

In the lower halves of Figures 1 and 2 are shown the daily outputs of potassium in urine and stools superimposed on the total daily intake of potassium. The upper part of each figure depicts the cumulative balance of potassium (corrected for nitrogen with an assumed K/N ratio of 2.7) and the serum potassium concentration. Tables I and II present the complete balance data from which these and subsequent figures are derived.

The two figures are alike in demonstrating a rapid steady rise in serum potassium concentration and cumulative potassium balance. During the first week large positive balances resulted from the

TABLE II—

R. S. 9 24		Intake							Urine						
Day	Body weight Kgm.	H_2O ml.	Na mEq.	K mEq.	Cl mEq.	N gms.	P gms.	Ca mgs.	Vol. ml.	Na mEq. per day	K mEq. per day	Cl mEq. per day	P gms. per day	NH_4 mEq. per day	N gms. per day
1	43.56	2,000	85	44	88	8.3	.57	90	1,930	55	3	45	.71	66	7.7
2	43.44	2,000	127	44	130	8.3	.57	90	1,450	62	3	42	.59	45	7.3
3	43.82	2,200	127	44	130	8.3	.57	90	1,450	72	4	54	.46	40	8.4
4	44.36	2,200	127	44	130	8.3	.57	90	1,420	63	4	48	.40	38	6.5
5	44.74	2,200	128	44	131	8.3	.57	90	1,440	68	4	56	.53	36	5.9
6	45.10	2,210	127	64	150	8.3	.57	90	1,550	92	6	78	.43	37	5.9
7	45.47	2,150	127	64	150	8.3	.57	90	1,494	97	6	85	.34	34	6.3
8	45.85	2,210	127	64	150	8.3	.57	90	1,675	92	6	85	.34	39	7.7
9	46.10	2,210	127	64	150	8.3	.57	90	1,870	124	8	116	.34	35	5.4
10	46.09	2,210	127	64	150	8.3	.57	90	2,080	171	10	152	.37	51	5.6
11	45.72	2,210	127	64	150	8.3	.57	90	2,550	249	14	213	.31	42	6.9
12	44.95	2,183	127	73	159	8.3	.57	90	2,743	268	24	240	.24	30	6.0
13	44.10	2,200	127	44	130	8.3	.57	90	3,235	319	34	294	.28	24	6.6
14	42.79	2,200	127	44	130	8.3	.57	90	2,410	207	43	180	.43	13	6.3
15	42.12	2,200	127	44	130	8.3	.57	90	2,210	192	55	160	.71	11	7.1
16	41.64	2,200	127	44	130	8.3	.57	90	1,700	139	60	122	.71	14	7.1
17	41.41	2,200	127	44	130	8.3	.57	90	1,360	80	51	85	.56	19	6.9
18	41.79	2,200	127	44	130	8.3	.57	90	1,710	67	49	77	.53	23	6.5
19	41.78	2,200	127	44	130	8.3	.57	90	1,350	62	36	71	.40	24	6.8
20	41.95	2,200	127	44	130	8.3	.57	90	1,680	84	34	92	.40	25	7.2

Analytical data

				Stool						Serum					
T.A. *mEq. per day*	Org. acids *mEq. per day*	Ca *mgs. per day*	pH	Na *mEq. per day*	K *mEq. per day*	Cl *mEq. per day*	N *gms. per day*	P *gms. per day*	Ca *mgs. per day*	pH	CO_2 *mM per l.*	Na *mEq. per l.*	K *mEq. per l.*	Cl *mEq. per l.*	P *mM per l.*
17	53	6	6.4	21	13	10	1.4	.16	221	7.4	28.0		2.1	94	
9	38	3	6.6	21	13	10	1.4	.16	221			141	2.2		
9	38	4	6.8	21	13	10	1.4	.16	221	7.5	30.5	139	2.2	92	
8	35	6	6.6	25	10	1	1.6	.25	226			139	2.3		
8	35	6	6.8	25	10	1	1.6	.25	226	7.4	28.0	141	2.4	94	0.90
6	34	9	6.8	25	10	1	1.6	.25	226				2.7		
7	34	14	6.9	13	8	7	1.2	.16	151	7.4	28.0	140	3.1	99	1.13
8	35	18	6.7	13	8	7	1.2	.16	151			148	2.9		
6	31	29	6.9	13	8	7	1.2	.16	151	7.4	27.5	145	3.2	101	1.37
8	30	56	6.7	13	8	7	1.2	.16	151			146	3.7		
9	29	66	6.7	13	8	7	1.2	.16	151				4.0		
7	33	66	6.9	13	8	7	1.2	.16	151	7.4	27.0		4.0	104	1.26
8	31	69	6.8	4	10	3	0.9	.12	159				4.2	102	
4	27	91	6.9	4	10	3	0.9	.12	159	7.4	25.5	141	4.6	102	1.52
8	38	73	6.8	4	10	3	0.9	.12	159			142	4.8		
12	38	49	6.9	4	10	3	0.9	.12	159	7.4	27.5		5.1	103	1.34
13	38	58	6.9	4	10	3	0.9	.12	159				5.0	104	
15	37	61	6.0	4	10	3	0.9	.12	159						
15	38	36	6.5	4	10	3	0.9	.12	159	7.4	26.0	140	4.4	105	1.45
												141	4.9		

low renal excretion of potassium. The average urine potassium in this period was only 4 mEq. per day in R. S. and 9 mEq. per day in A. B., despite the fact that both subjects excreted approximately 1 to 2 l. of urine per day containing 6 to 8 gms. of total nitrogen.

In A. B. (Figure 1), urinary excretion rose rapidly after the eleventh day, resulting in an abrupt termination of the potassium-retention phase. Potassium uptake was complete after 13 days when A. B. had retained a total of 536 mEq. in excess of nitrogen (12 mEq. per kgm. of final weight). At the same time serum potassium concentration reached normal levels. Thereafter, urine output of potassium kept pace with intake and there was no further significant change in serum potassium or external potassium balance.

The course of events in R. S. was quite similar to that in A. B. except that retention of potassium continued until serum potassium had reached abnormally high levels. Serum drawn a few hours after the evening meal on the twelfth day had a

Analytical data

				Stool						Serum					
T.A. *mEq. per day*	Org. acids *mEq. per day*	Ca *mgs. per day*	pH	Na *mEq. per day*	K *mEq. per day*	Cl *mEq. per day*	N *gms. per day*	P *gms. per day*	Ca *mgs. per day*	pH	CO_2 *mM per l.*	Na *mEq. per l.*	K *mEq. per l.*	Cl *mEq. per l.*	P *mM per l.*
17	32	56	6.5	4	6	2	1.2	.37	170	7.43	30.0	147	2.7	100.5	1.42
12	34	30	6.6	4	6	2	1.2	.37	170				2.2		
9	31	35	6.8	4	6	2	1.2	.37	170	7.47	33.5		2.8	101.0	0.97
9	32	34	6.7	4	6	2	1.2	.37	170				3.3		
11	33	33	6.7	4	6	2	1.2	.37	170	7.46	32.5	146	3.2	102.0	1.26
10	36	46	6.8	4	6	2	1.2	.37	170	7.47	32.0	144	3.3	102.0	
8	34	57	6.6	4	6	2	1.2	.37	170			147	3.5		1.29
2	37	35	7.2	4	6	2	1.2	.37	170	7.43	28.0	145	3.2	107.0	1.35
4	30	54	6.9	13	6	7	0.8	.12	83			147	4.0	108.0	1.31
none	37	71	7.7	13	6	7	0.8	.12	83	7.45	29.0	147	4.0	107.0	1.32
none	39	78	7.7	1	2	1	0.2	.06	35						
none	35	70	7.6	1	2	1	0.2	.06	35	7.45		146	5.0	105.0	1.32
4	36	76	7.3	1	2	1	0.2	.06	35	7.45	28.0	145	5.6	108.0	1.41
11	34	33	6.7	1	2	1	0.2	.06	35			145	5.6	105.0	1.36
13	33	51	6.6	1	2	1	0.2	.06	35	7.45	27.5	146	6.1	105.0	1.34
16	32	51	6.5	1	2	1	0.2	.06	35			144	5.6	107.0	
20	35	46	5.7	1	2	1	0.2	.06	35	7.44	25.0		5.2	107.0	1.52
21	34	72	5.7	1	2	1	0.2	.06	35						
17	35	41	5.7	1	2	1	0.2	.06	35	7.42	26.0	143	4.3	108.0	1.52
19	34	60	5.3	1	2	1	0.2	.06	35		26.0			107.0	

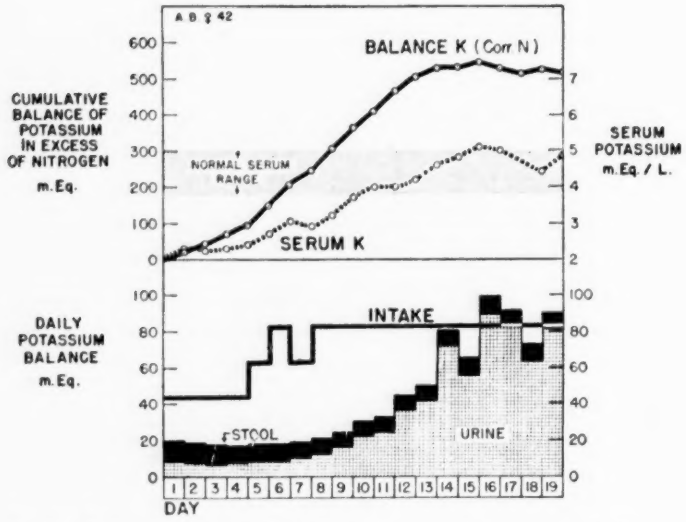

FIG. 1. SERUM POTASSIUM, CUMULATIVE POTASSIUM BALANCE, AND DAILY EXTERNAL BALANCE OF POTASSIUM, PATIENT A. B.

potassium concentration of 7.5 mEq. per l. At this time, electrocardiographic tracings showed evidences of hyperkaliemia and it was deemed advisable to reduce the patient's oral intake of potassium from 73 to 44 mEq. per day in order to avoid further intoxication. On the fifteenth to eighteenth days of the study urinary excretion exceeded intake and the cumulative retention of potassium in excess of nitrogen gradually declined to a final level of roughly 445 mEq. (10.8 mEq. per kgm. of final weight), while the serum concentrations returned to normal.

B. *Total exchangeable potassium*

In A. B. total exchangeable potassium was determined at the beginning and end of the balance

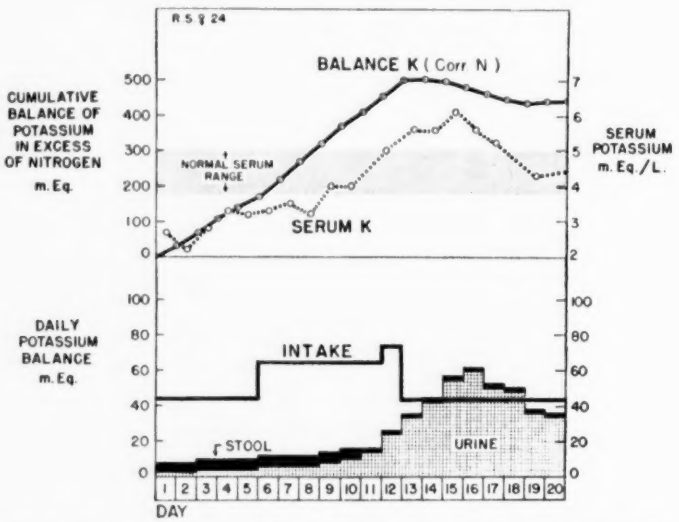

FIG. 2. SERUM POTASSIUM, CUMULATIVE POTASSIUM BALANCE, AND DAILY EXTERNAL BALANCE OF POTASSIUM, PATIENT R. S.

TABLE III

Correlation of potassium balance and exchangeable potassium, patient A. B.

Time	Exchangeable K *mEq.*	Change in exchangeable K *mEq.*	Change in K balance *mEq.*
Day 0	1,120	—	—
Day 19	1,659	+539	+548

period. These values are compared in Table III with the observed change in potassium balance. It will be seen that, at the beginning of the study, this patient's total exchangeable potassium was only 1120 mEq., or 25.2 mEq. per kgm. (Mean normal for women by this technique is 41 mEq. per kgm. [15].) At the end of the balance this figure was 1659 mEq., or 37.3 mEq. per kgm. This increase of 539 mEq. is almost identical with the observed total cumulative retention of 548 mEq. over the same period.

In R. S. the first determination of exchangeable potassium was made on the sixth day of study. Correcting the observed figure of 1425 mEq. for the over-all retention of potassium prior to, and subsequent to, this determination, one derives an estimate for initial exchangeable potassium of 27.0 mEq. per kgm. and a final figure of 40.4 mEq. per kgm. Several weeks after this study was completed, total exchangeable potassium, determined on two occasions, averaged 39.4 mEq. per kgm.

In each instance, therefore, initial body potassium content was far below normal limits, but was restored to normal at the end of the balance. The potassium deficit was approximately one-third of the normal total exchangeable potassium content.

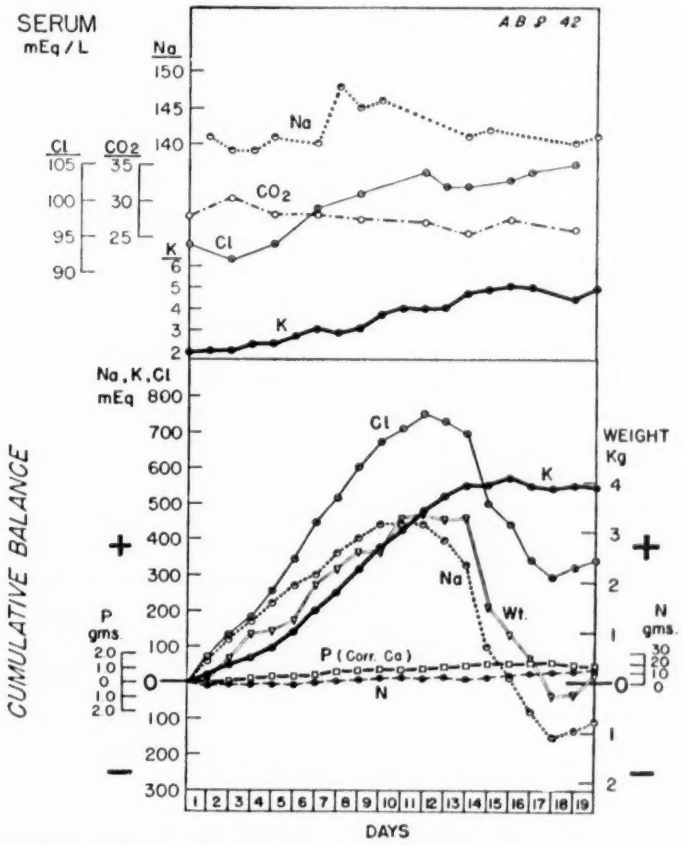

FIG. 3. CUMULATIVE ELECTROLYTE AND NITROGEN BALANCE, WEIGHT CHANGES, AND SERUM ELECTROLYTES, PATIENT A. B.

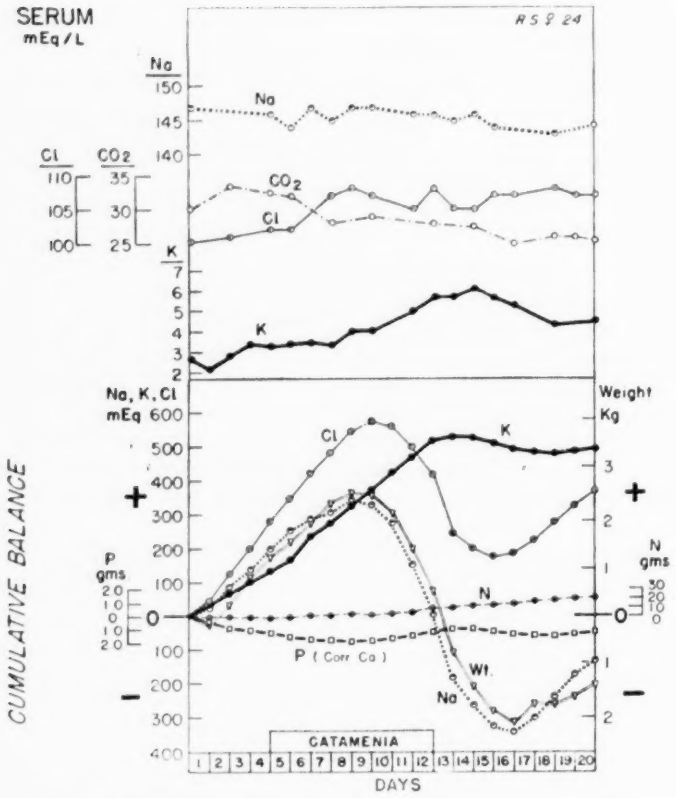

FIG. 4. CUMULATIVE ELECTROLYTE AND NITROGEN BALANCE, WEIGHT CHANGES, AND SERUM ELECTROLYTES, PATIENT R. S.

C. *Serum electrolytes and pH*

The upper sections of Figures 3 and 4 show the values for serum electrolyte concentrations. Both subjects initially had a marked hypokaliemia which improved gradually as described in Section A. Sodium concentrations were normal in both subjects and did not change significantly. Serum chloride concentration was a little low in A. B., but only in R. S. (Figure 4) was there a slight increase in initial serum bicarbonate. As potassium balance was restored in this patient, serum bicarbonate concentration returned to normal. In A. B. (Figure 3), although serum bicarbonate fell only 2 mEq. per l., chloride concentration rose 10 mEq. per l. This suggests the disappearance from the serum of some unmeasured anion, possibly an organic acid, which had been present during the potassium-depleted state. Serum pH (Tables I and II) was always within normal limits and did not vary significantly as potassium was accumulated.

D. *Electrolyte balances and weight changes*

In the lower halves of Figures 3 and 4 are plotted cumulative changes in body weight and in external balance of electrolytes and nitrogen. The scale for the cumulative weight change is so chosen that one kilogram on the weight scale is equivalent on the electrolyte scale to 140 mEq. (This latter value was the approximate mean sodium concentration in extracellular fluid.) When changes in weight are due solely to changes in extracellular fluid volume the lines indicating sodium balance and weight should be parallel, provided that extracellular sodium concentration remains the same. Unmeasured skin losses would cause deviation of the weight and the sodium curves. Little or no sweat-

ing occurred during these experiments so that unmeasured losses of sodium through the skin were probably only 5 to 10 mEq. per day (16).

During the initial phase of potassium repletion there was a striking retention of sodium and chloride which was followed by rapid diuresis of these ions just as potassium retention was approaching completion. In R. S. retention of sodium occurred just prior to and during menstruation, but A. B., who was not menstruating, showed similar retention of sodium.

The cumulative weight curve in each subject was closely related to the change in extracellular fluid volume predicted from sodium balance, and the close adherence of the two curves would suggest that expansion and contraction of extracellular fluid volume was responsible for the changes in weight. The curve of chloride balance paralleled that of sodium, but chloride retention always greatly exceeded sodium retention. On the thirteenth day in R. S. (Figure 4) and on the fifteenth day in A. B. (Figure 3), when the cumulative changes in weight and sodium balance were approximately zero, it should be noted that there were positive balances of potassium and chloride of approximately 500 mEq. each. This retention occurred without significant change in body fluid tonicity as measured by the sum of the concentrations of extracellular sodium and potassium.

E. *Calcium, phosphorus and nitrogen balance*

The scales for nitrogen and phosphorus balances in Figures 3 and 4 are related to each other and to the scale for electrolytes so that 1 gm. of phosphorus equals 14.7 gms. of nitrogen or 39.7 (14.7 × 2.7) mEq. of potassium. These are the approximate relations of these substances in protoplasm (17) and parallel curves for nitrogen, phosphorus, and potassium are therefore presumed to indicate that changes in the balance of these substances are due to gain or loss of tissue.

It is immediately obvious from a consideration of the figures that nitrogen retention was small and the retention of potassium was far in excess of the retention of nitrogen.

Using a Ca/P ratio for bone of 2.23 (17), the phosphorus balances plotted in Figures 3 and 4 have been corrected for calcium balances (Tables I and II). The small negative balances of calcium probably were the result of the relatively low calcium intake (18). The cumulated corrected phosphorus retention for A. B. (Figure 3) exceeds the retention predicted from nitrogen balance by + .54 gm. This is within the limits of error in analysis and balance technique (17). In R. S. (Figure 4) the corrected phosphorus balance was 2.6 gms. less than that predicted from the nitrogen balance. This may indicate a small loss of phosphorus from cells. In any event, it is quite clear from these figures that potassium retention occurred with little or no change in the cell balance of phosphorus.

TABLE IV

Internal balances

Subject	Intracellular sodium *mEq.*	Intracellular potassium *mEq.*
A. B. Days 1–15	−483	+505
R. S. Days 1–14	−454	+462

F. *Cell balances of sodium and potassium*

Shifts of sodium and potassium to and from intracellular fluid at the end of the two-week period

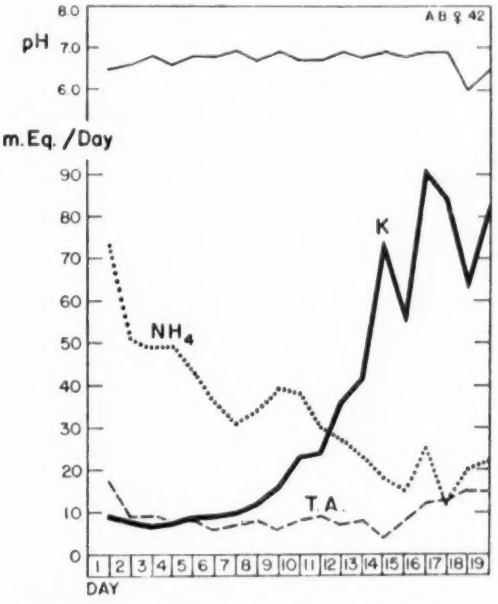

FIG. 5. RENAL EXCRETION OF AMMONIUM, TITRATABLE ACID AND POTASSIUM, AND URINE pH, PATIENT A. B.

of potassium repletion are shown in Table IV. The similarity of the magnitude of the shifts at the time of complete repair in both subjects and the almost equal exchange of sodium for potassium are striking. In R. S. it is estimated that 462 mEq. of potassium entered cells in exchange for 454 mEq. of sodium. In A. B. 483 mEq. of sodium left cells while 505 mEq. of potassium were taken up.

G. *Red blood cell composition*

Initial concentrations of sodium, potassium and phosphorus, expressed either as "per kgm. of red blood cell solids" or "per kgm. of red blood cell water," were both within normal limits (19). During potassium repletion there was a rise in each patient of approximately 13 per cent in red cell potassium content, but the final values were still normal. Red cell phosphorus did not change significantly.

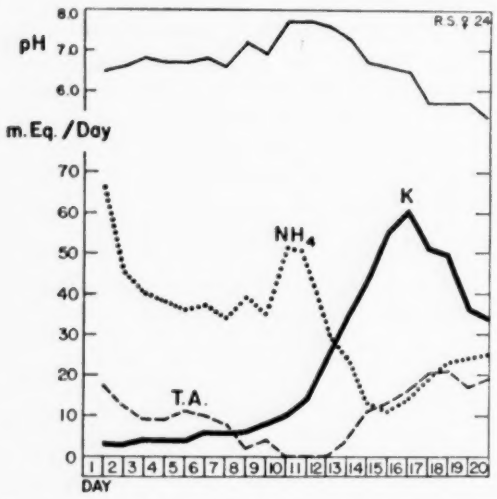

FIG. 6. RENAL EXCRETION OF AMMONIUM, TITRATABLE ACID AND POTASSIUM, AND URINE pH, PATIENT R. S.

(The ammonium excretion for the eleventh day has been plotted incorrectly. It should be 42 mEq.)

H. *Urine pH, excretion of ammonium, titratable acid, and organic acids*

The changes in urine pH, ammonium, and titratable acid, and the relation of these values to urinary potassium are shown in Figures 5 and 6.

TABLE V*

Renal function data, patient R. S.

Patient R. S. ♀ 24	Before K therapy 10/2/51	After K therapy 11/6/51	11/29/51	2/7/52
C_{In} ml./min.	—	103	99	116
C_{Cr} ml./min.	—	88	111	133
P_{Cr}† mgm. %	—	.82	.76	.71
C_{PAH} ml./min.	—	379	462	507
FF %	—	27.3	21.4	22.9
Hkt %	—	37.4	39.0	42.5
ERBF‡ ml./min.	—	606	758	882
Tm_{PAH} mgm./min.	—	74	90	93
Sp. Gr.	1.013	1.013	—	1.026

* All clearance and Tm_{PAH} values corrected to 1.73 sq. m.
† Serum creatinine concentration.
‡ Effective renal blood flow, calculated from C_{PAH} and Hkt.

An outstanding feature of each figure is the progressive decline in excretion of ammonium. In A. B. (Figure 5), ammonium decreased without significant alteration in urine pH or titratable acid. In R. S. (Figure 6), the sharp decline in urinary ammonium which began on the twelfth day was associated with a fall in urine pH and a rise in titratable acidity.

Except for the period of the initial fall in ammonium excretion when the balance regime was started, there was a roughly inverse relationship between excretion of ammonium and potassium.

Excretion of total organic acids did not change significantly at any time in either patient.

TABLE VI*

Renal function data, patient A. B.

Patient A. B. ♀ 42	Before K therapy 1/16/52	After K therapy 2/5/52	3/21/52	Following diarrhea 7/15/52
C_{In} ml./min.	43	72	103	73
C_{Cr} ml./min.	56	88	125	87
P_{Cr} mgm. %§	.87	.94	.70	.79
C_{PAH} ml./min.	315	376	584	346
FF %	14.0	19.2	17.7	21.0
Hkt %	38	29	35	35
ERBF† ml./min.	508	530	897	531
Tm_{PAH} mgm./min.	45	53	71	69
E_{PAH}	—	.71	—	—
RBF‡ ml./min.	—	746	—	—
A-V O_2 vols. %	—	1.33	—	—
R O_2 ml./min.	—	9.9	—	—
Sp. Gr.	1.005	1.015	1.025	—

* All clearances, Tm_{PAH} and R O_2 corrected to 1.73 sq. m.
† Effective renal blood flow, calculated from C_{PAH} and Hkt.
‡ True renal blood flow, calculated from ERBF and E_{PAH}.
§ Serum creatinine concentration.

II. *Renal Function Studies*

The renal function data are presented in Tables V and VI. All clearances and Tm values are corrected to a standard surface area of 1.73 sq. m.

1. *Patient R. S. (Table V):*

The first observations were made approximately a week after this patient's potassium deficit had been corrected. C_{In} and Tm_{PAH} were at the lower limits of normal, but C_{Cr} and C_{PAH} were slightly below normal. Prior to restoration of potassium balance as well as immediately thereafter, dehydration for 18 hours had resulted in a maximum urine specific gravity of only 1.013.

During the subsequent months, the patient remained well and her serum potassium concentration and electrocardiographic records did not show any evidence of recurrence of potassium depletion. Three weeks after the first observations C_{Cr} and C_{PAH} were at the lower limits of normal and Tm_{PAH} had increased from low normal to high normal values. Final observations made 10 weeks later (14 weeks after the restoration of potassium balance) revealed that all renal functions were unequivocally normal. As indicated in Table V, C_{Cr}, C_{In}, and C_{PAH} increased slightly, but Tm_{PAH} remained at its previous high normal level. The patient was now able to concentrate her urine to 1.026.

2. *Patient A. B. (Table VI):*

A. B.'s renal function was first measured immediately before restoration of her potassium deficit was begun. C_{In} was 43 ml. per min. and C_{Cr} was 56 ml. per min. Tm_{PAH} was also greatly reduced and C_{PAH} was little more than half of normal. Maximum urine specific gravity was only 1.005.

The second observation was made immediately after body potassium had been completely restored. At this time all functions measured were considerably improved, but nevertheless they were still far below normal. Renal A-V O_2 difference was normal, but renal oxygen consumption was 9.9 ml. per min. (normal about 16 ± 2.9 ml. per min. [20]), and E_{PAH} was only .71. True renal blood flow (RBF) was therefore considerably higher than the effective blood flow (ERBF) and was near the lower limit of normal. Maximum specific gravity was 1.015.

During the next six weeks the patient remained well, and occasional electrocardiographic tracings and serum potassium determinations were normal. At the end of this time, a third evaluation of renal function revealed that all functions measured (C_{In}, C_{Cr}, C_{PAH}, Tm_{PAH}) had increased substantially and were now within normal limits. A concentration test produced a maximum urine specific gravity of 1.025.

The final study of renal function listed in Table VI was carried out at the end of a two-week period of diarrhea, when the serum potassium was 3.0 mEq. per l. and the electrocardiogram showed marked changes in Q-T interval and T-waves consistent with hypokaliemia. C_{Cr}, C_{In}, and C_{PAH} were now roughly 30 per cent below their previously normal levels, but Tm_{PAH} was essentially unchanged.

DISCUSSION

That excessive use of laxatives was the sole cause of the potassium depletion in these two women is not proven conclusively by these data, but the clear-cut history of diarrhea related to the chronic use of laxatives and the lack of evidence for any other mechanism make this conclusion highly probable. Furthermore, when R. S. was allowed to use laxatives again, balance study demonstrated loss of potassium in the diarrheal stools and a negative daily potassium balance (21). The concentration of potassium in stool water during this laxative-induced diarrhea was 50 to 55 mEq. per l., and the stools contained 2 to 4 per cent of solids.

The extraordinarily severe potassium deficits in these two women apparently developed gradually over a period of months or years without producing any striking symptoms or signs. This suggests that the rate at which potassium depletion develops is of importance in determining symptomatology. Although rapid loss of potassium from isolated perfused muscle will reduce contractility (22), it has been shown that the gradual production of potassium depletion in rats does not impair the muscular response to a tetanic stimulus (23) or reduce the animals' swimming ability (24). Further evidence that tissue may become acclimatized to potassium deficiency is provided by the "potassium paradox" of Libbrecht (25).

The important contribution of renal "leakage"

of potassium to the development of potassium depletion in man has been emphasized (*26, 27*). Although there are no observations on the efficiency of renal conservation of potassium early in the development of the potassium deficit in the two cases reported here, the data in R. S. demonstrate unequivocally that, with prolonged depletion, renal excretion of potassium may sometimes be reduced to extremely low levels despite normal urine volumes. In this patient the renal excretion of potassium during repair remained so low that her body fluids became temporarily surfeited with this ion and signs of potassium intoxication appeared in the electrocardiogram. This temporary failure in renal regulation may have been due in part to impairment of renal function.

The very close correlation between total exchangeable potassium and the potassium balance in patient A. B. is noteworthy. This observation implies that exchangeable potassium measured the metabolically active pool of potassium in the body, irrespective of whether it measured total body potassium.

The apparent discrepancy between the net balance of water and cations (Section D, above) could have resulted from excessive unmeasured skin losses of electrolytes or from other systematic errors in balance technique. However, in the absence of any evidence for such an explanation, it would appear likely that uptake of potassium was associated with a reduction in the osmotic activity of some intracellular constituent (*28, 29*). Total intracellular cation concentration (calculated on the assumptions that [*a*] "chloride space" is equivalent to extracellular space, and [*b*] intracellular calcium and magnesium did not change [*29*]) increased approximately 40 mEq. per l. in R. S., and 25 mEq. per l. in A. B. If the final tissue composition was normal, this would indicate a low intracellular cation concentration prior to treatment. Similar changes are regularly found in the muscles of potassium-deficient rats (*29*).

Restoration of tissue potassium was also accompanied by a large but transient retention of sodium, an observation similar to that made by Elkinton, Squires, and Crosley (*30*). Sodium retention may have been related to the reversible disturbances in renal function observed in the present patients. No explanation can be offered at present for the sustained retention of chloride (Figures 3 and 4) and expansion of the "chloride space."

The absence of significant alkalosis in these patients despite a prolonged period of severe potassium depletion requires consideration. It has been suggested that the alkalosis usually present in potassium deficiency may be due, at least in part, to exchange of intracellular potassium for extracellular hydrogen ions (*31, 32*). The approximate equivalence of the sodium and potassium shifts listed in Table IV would suggest that no large transfers of hydrogen occurred in either of the present cases. However, the methodological errors inherent in these calculations (*31*) preclude close interpretation of the data. Further evidence against a shift of hydrogen ions from the cells can be deduced from the fact that the excretion of ammonium plus titratable acid decreased during the uptake of potassium. This is in contrast to the findings of Cooke and associates (*32*), who observed an increased excretion of ammonium and titratable acid during treatment of potassium-deficient, alkalotic rats. Interpretation of the present data is complicated by the possibility that previous loss of bicarbonate in the diarrheal stools may have affected the acid-base balance.

The observations of urinary excretion of ammonium and potassium depicted in Figures 5 and 6 show that, contrary to prevailing hypotheses (*33*), changes in ammonium excretion were independent of changes in urine pH. The rapid decline in urinary ammonium coincided with an increase in the excretion of potassium and sodium in both subjects. Such an inverse relationship between the urinary excretion of potassium and ammonium might be expected if renal ammonium production involves secretion of hydrogen ions (*33*) and if there is a competition between tubular secretion of hydrogen and potassium (*34*). However, the hypothesis of hydrogen-potassium competition would still fail to explain the apparent indifference of urine pH and titratable acidity to the large changes in potassium excretion.

An alternative explanation is that ammonium may, under certain conditions, passively serve the demands of electroneutrality in the urine by acting as "available cation." According to this hypothesis the presence of increasing amounts of potassium ions in the tubular fluid would, if all other relevant factors were kept constant, reduce the stimu-

lus for release of ammonia by reducing the potential excess of urinary anions over cations. However, in view of the evidence presented here that potassium depletion impairs certain tubular functions, any interpretation of these data must be considered for the present as only tentative.

The unexpected discovery of fixed urine specific gravity in patient R. S. while she was potassium-depleted suggested that more detailed study of her renal function might be of interest. It was not until after balance studies had been completed and potassium balance restored that the clearances reported in Table V could be carried out. The changes in renal function in this patient were small but they suggested that glomerular and tubular function may have been impaired during the period of potassium depletion.

The more complete observations in patient A. B. who also initially exhibited isosthenuria indicated that there was marked impairment of glomerular and tubular function during potassium depletion which slowly returned to normal after treatment with potassium. Immediately after a second episode of diarrhea and potassium depletion renal function was found once more to be distinctly impaired.

Both patients had habitually ingested laxative mixtures containing aloin. Although this anthraquinone compound is said to cause renal damage in rabbits when administered subcutaneously in total doses of 0.2 to 2.25 gms. over a period of 1 to 40 days (35), the quantities of aloin ingested by these patients over a comparable period were far below this toxic level. Furthermore, humans are resistant to the nephrotoxic action of aloin because they excrete practically none of this substance in the urine when the drug is taken by mouth (36).

Disorders of fluid and electrolyte metabolism known to impair renal function, such as dehydration, sodium depletion, and alkalosis, were not present in these patients. The absence of any concomitant weight gain or retention of nitrogen suggests that the improvements in renal function cannot be ascribed to improved nutrition. Recent serial studies of another patient with severe chronic potassium depletion have also revealed reductions in glomerular and tubular function which were restored within six weeks by treatment with potassium (21). Although this patient's potassium depletion was complicated by steatorrhea and malnutrition, there was no clinical change in these factors during the period of observation, and there were no other known disorders of water or electrolyte balance. The present data would therefore strongly suggest that potassium depletion may depress renal function.

Tubular dysfunction in these patients may be related to the observations of altered renal tubular morphology in potassium-deficient rats (37) and in patients dying with clinical evidences of potassium depletion (38, 39). The apparent changes in renal plasma flow could have been due in part to reductions in the tubular extraction of PAH such as occurred in patient A. B. (See Table VI.) In addition, plasma flow, as well as glomerular filtration, may have been affected by the expansion of the "chloride space" which occurred during uptake of potassium (Figures 3 and 4).

Renal dysfunction of the type reported here could account for certain phenomena associated with severe potassium depletion. Sodium retention (30, 40, 41), elevated blood nitrogen concentration (40), and reduced urea clearance (41) may be in part due to the reduction in glomerular filtration. Renal tubular damage could explain the isosthenuria in these and other cases of potassium depletion (38, 39, 42) as well as the polyuria in potassium-deficient dogs (43) and rats (44). The failure of the kidney in potassium depletion to adjust extracellular bicarbonate concentration (32) may also be related to the functional disorders reported here. Another interesting implication of these data is that potassium depletion may be responsible at least in part for the renal dysfunction found in many cases of alkalosis (30, 45).

SUMMARY AND CONCLUSIONS

Balance studies were carried out on two women who had gradually developed severe potassium depletion and hypokaliemia as the result of chronic diarrhea induced by overuse of laxatives. Neither of the patients had any overt neuromuscular symptoms or signs, and it was only through the discovery of T-wave changes in routine electrocardiograms that hypokaliemia was discovered. There were no other disturbances of serum electrolytes except for slight elevation of plasma bicarbonate in one patient. Red cell sodium, potassium and

phosphorus concentrations were normal. Renal excretion of potassium was very low.

When laxatives were withdrawn and the patients given normal oral intakes of potassium, each retained an amount of potassium roughly equivalent to one-half her total initial body potassium content (as estimated by K^{42} dilution), without significant retention of nitrogen or phosphorus. Changes in total exchangeable potassium were approximately equal to the actual potassium retention. In both instances potassium retention was accompanied by a large but transient retention of sodium. Internal balance calculations indicated that cellular uptake of potassium was approximately equal to the estimated loss of intracellular sodium and that total initial intracellular cation concentration was markedly reduced. Excretion of ammonium was relatively high initially and during correction of the potassium deficit ammonium diminished rapidly without significant change in urine pH or titratable acidity.

Ability to concentrate the urine was impaired in both patients prior to treatment, but was restored to normal after correction of the potassium deficit. Clearance studies begun immediately after treatment in the first patient revealed slight reductions in glomerular and tubular function. In the second patient glomerular and tubular functions were found to be markedly depressed prior to therapy. After correction of the potassium deficit there was a gradual return to normal of all functions in both patients. It is suggested that renal dysfunction may account for certain disturbances in water and electrolyte metabolism observed in potassium deficiency.

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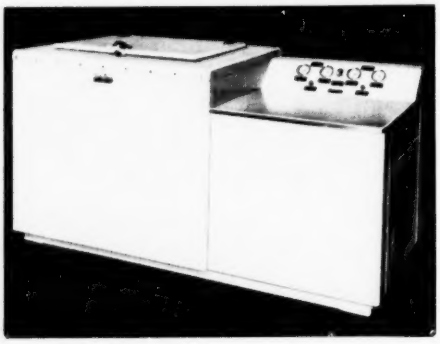

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